

INTERPHASE SOLUBILITY AND
CHROMATOGRAPHIC RETENTION

By

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This dissertation is dedicated
to my fiance, Daniel Coffman.
I could never have done
this without you.

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The retention and selectivity behavior of small solutes on silica and controlled pore glass (CPG) reversed phase liquid chromatographic (RPLC) stationary phases was studied as a function of stationary phase alkyl bonding density. These monomeric octadecyl phases were synthesized by both reflux and ultrasound methods; high alkyl bonding densities ($3.60 \mu\text{mol}/\text{m}^2$) were obtained via low temperature ultrasound reactions using 4-dimethylaminopyridine as the acid-acceptor catalyst. Using an improved method for the calculation of the stationary phase volume, the chromatographic capacity factors for the solutes were divided by the volume phase ratio (stationary/mobile) to obtain the thermodynamic partition coefficients; their behavior as a function of stationary phase octadecyl bonding density was examined in

55/45 methanol/water and 85/15 acetonitrile/water mobile phase systems.

For the silica packings in both mobile phase systems, the partition coefficients linearly increased until a critical bonding density of about $3.1 \mu\text{mol}/\text{m}^2$ was reached; after this point the partition coefficients began decreasing with increasing bonding density. This behavior supports a partitioning retention mechanism for RPLC. In this model, the driving force for retention is the creation of a solute sized cavity in the stationary phase interphase structure. Beyond the critical density, increased alkyl bonding density results in enhanced interphase chain packing constraints which increase the energy necessary for solute cavity formation, resulting in decreased chromatographic partition coefficients.

Methylene and phenyl selectivity were also examined as a function of octadecyl bonding density. Methylene selectivity was approximately constant, but phenyl selectivity increased linearly with bonding density. This further supports the partitioning theory; methylene selectivity is not expected to be affected by chain ordering but phenyl selectivity for the linear solutes used should increase as the interphase packing structure becomes more ordered. Identical selectivity and retention studies on the CPG bonded phases garnered inconclusive results, as no obvious trends were discernable in either study.

This work supports a partitioning mechanism for RPLC retention and as such gives insight into the retention

process on a molecular level. This theory is predictive without adjustable parameters and is relevant to partitioning behavior in organized assemblies, including micelles, membranes and vesicles.

CHAPTER I INTRODUCTION

Models of Reversed Phase Liquid Chromatographic Retention Retention Indices Based on Solute Descriptors

Reversed phase liquid chromatography (RPLC) is one of the most popular and powerful analytical separation methods. In RPLC, the stationary phase support consists of silica particles which are typically 3 to 10 μm in diameter; alkyl chains, usually 8 or 18 carbons in length, are attached to oxygen atoms on the silica surface via covalent bonds, resulting in a nonpolar surface. The mobile phase consists of water and an organic modifier such as methanol, acetonitrile or tetrahydrofuran. Thus the mobile phase is much more polar than the stationary phase; mobile phase polarity is adjusted by varying the volume ratios of water and organic modifier. It has been estimated that 80-90% of the high performance liquid chromatography (HPLC) systems currently in use are reversed phase systems (Melander and Horvath, 1980). Yet many practitioners of RPLC view this technique as "black magic" because its retention mechanism is not well understood, especially at the molecular level. This makes the prediction of retention for new compounds of interest extremely difficult. A basic understanding of RPLC retention at the molecular level is a necessity for the

formulation of a predictive retention index system. Such a retention index system would allow accurate interlaboratory comparison of RPLC retention data.

Chromatographic retention is most often quantified by the capacity factor, k' . Thermodynamically, the capacity factor for a chromatographic solute is the ratio of the number of moles of solute in the stationary and mobile phases. The capacity factor is also a measure of solute retention which normalizes for the mobile phase flow rate and the physical dimensions of the chromatographic column, since $k' = (V_r - V_m)/V_m$, where V_r is the solute retention volume and V_m is the retention volume of an unretained solute, often called the dead volume. Many different investigators have attempted to correlate RPLC retention data with topological, geometric and/or calculated physical property descriptors of chromatographic solutes in order to predict RP retention. Topological descriptors include molecular connectivity, molecular complexity and correlation factor; van der Waals volume, molecular surface area and length/breadth parameters are geometric descriptors. Physical property descriptors include hydrophobic substituent constants, UNIFAC models of activity coefficients, and octanol/water partition coefficients (D'Amboise and Bertrand, 1986; Funasaki et al., 1986; Jinno and Kawasaki, 1984a, 1984b and 1984c; Petrovic et al., 1985).

Topological descriptors such as molecular connectivity indices are used to correlate chromatographic retention with molecular structure. These indices are numerical values which quantitatively describe carbonaceous adjacency relationships in the molecular structure of a solute (Lehtonen, 1984). Molecular connectivity indices have been shown to be proportional to the cavity surface area of a molecule. When a nonpolar hydrocarbon solute is introduced into an aqueous or hydroorganic environment, a large negative entropy of solution results. It has been suggested that this negative entropy is a result of structural ordering around the hydrocarbon molecule (Karger et al., 1976). This ordering comes about from the formation of a cavity of water molecules around the hydrocarbon molecule. To overcome the entropy loss, nonpolar molecule segments will try to remove themselves from the aqueous medium and/or they will group together. The term "hydrophobic effects" is used to describe these two phenomena of cavity formation and nonpolar clustering. The calculated surface area of the water cavity is significant because it can be related to the solubility of hydrocarbons in water (Karger et al., 1976). Likewise molecular connectivity, since it is also proportional to the cavity surface area, has also been correlated to non-electrolyte water solubility; however in contrast to cavity surface area, simple first order molecular connectivity indices are quite easy to calculate. Since in some cases the logarithm of the aqueous solubility

of a solute is proportional to the logarithm of its capacity factor k' , it was expected that molecular connectivity would also be proportional to k' , allowing prediction of retention to be made from these molecular connectivity calculations (Karger et al., 1976).

In their comparisons of experimental capacity factors to those predicted via simple molecular connectivity calculations, Karger et al. (1976) found very good agreement for para-substituted phenols and primary alcohols. However, the predicted k' values were uniformly high for secondary alcohols. This is because the experimental $\log k'$ values for two of the primary alcohols were used to determine the slope and intercept in the presumed linear relationship between $\log k'$ and molecular connectivity index for both types of alcohols. The high predicted k' values for the secondary alcohols reflect that their steric environment is different from that of the primary alcohols, showing that simple molecular connectivity indices can only be used to predict relative retention for compounds with the same functional group as those used for standards (Karger et al., 1976).

Lehtonen (1984) used molecular connectivity indices of different orders, which correct for complex branching as well as for the nature of atoms other than carbon which make up the solute framework, to predict retention behavior for 16 dansylamides. Predicted and experimental k' values could be correlated very well by combinations of different order

connectivity indices. However, a computer program had to be used in order to find the best combination of indices to obtain good correlation and these combinations were often nonlinear, involving combinations of connectivity indices raised to powers ranging from -2 to +2. The choice of indices also varied according to which organic modifier was used in the mobile phase as well as its percent composition. Although Lehtonen obtained good correlations between predicted and experimental retention, his method requires extensive computer calculations as no general connectivity index combination was applicable even within the same class of solutes (dansylamides). These two types of examples point out the shortcomings in the use of molecular connectivity indices to predict retention: the capacity factors for at least two members of the same class of compounds must be determined in order to find the proportionality constant between capacity factor and molecular connectivity, these predictions are only valid for compounds of the same functional group as the standards, for complex molecules a computer program must be used in order to find the best combination of indices to predict retention and this combination for a particular type of molecule may change if the mobile phase composition is altered. This method is also unable to distinguish between geometric isomers (Funasaki et al., 1986).

Molecular complexity is a topological descriptor and the general index of molecular complexity (GIMC) is an index

whose applicability is general and which does not require the use of experimental or empirical data (D'Amboise and Bertrand, 1986). The GIMC is derived from combinations of graph theory and statistical information theory. It is so named because it considers all of the features which make a molecule more or less complex such as size, symmetry, branching, ring structures, multiple bonds and atomic heterogeneity. Molecules are represented by their skeletal molecular graph whose complexity is determined from a statistical information theory derived formula. Since any observable behavior related to a molecule's complexity is a function of the GIMC, chromatographic retention should also correlate with the GIMC. D'Amboise and Bertrand (1986) point out that GIMC is able to make retention predictions for solutes such as alcohols or fatty acids, which are not well correlated with hydrophobicity. They point out that GIMC is a structure sensitive parameter representing the various reactive attributes of a molecule; therefore it should be related to the interaction mechanism in retention. However, plots of $\log k'$ versus GIMC for alcohols show distinct curvature, especially for alcohols with five or less carbons. Other difficulties exist as well. The index does not seem to be well applicable to molecules with different heteroatoms that are similarly bonded or for molecules belonging to nonhomologous series. Correlation of data between different stationary phases has also proven to be a problem (D'Amboise and Bertrand, 1986).

Geometric descriptors are in general fairly easy to calculate. Van der Waals volume and surface area are both calculated from the van der Waals radii of the atoms from which the molecule is composed (Jinno and Kawasaki, 1984a). Length to breadth ratio (L/B) is a shape parameter based on the rectangle with minimum area which could envelop a molecule (Wise et al., 1981). However, all of these descriptors considered alone or in combination were found to have poor direct correlation with RPLC retention for substituted benzene derivatives. Jinno and Kawasaki (1984a) concluded that this indicates that molecular size and shape were not the dominant forces controlling retention for these molecules. However, they noted that size and shape are important contributors to retention for alkylbenzenes and polycyclic aromatic hydrocarbons (PAHs) (Jinno and Okamoto, 1984). Wise et al. (1981) have also found that L/B is useful for predicting PAH elution order; however this parameter is useless for establishing general PAH retention indices since these indices vary according to the type of octadecyl bonded phase column used, necessitating the determination of a retention index equation for each different octadecyl column.

Physical property descriptors have been the most successful for predicting RPLC solute retention. The physical properties on which these descriptors are based come about from the solute's solution behavior in the mobile and stationary phases (Karger et al., 1976). Since

retention is controlled by the thermodynamic equilibrium of the solute between the mobile phase and stationary phase, retention could theoretically be predicted from standard Gibbs free energies since $\Delta G^\circ = -RT \ln K$, where ΔG° is the standard Gibbs free energy, R is the gas constant, T the absolute temperature and K the equilibrium distribution constant for the solute between the stationary and mobile phases. Since $k' = K(V_s/V_m)$ where V_s and V_m are the stationary and mobile phase volumes, the capacity factor for a solute (and thus its normalized retention) could easily be calculated were ΔG° , V_s and V_m known. Since experimental ΔG° values for these systems are unavailable, they are often estimated using liquid mixture models with readily available physical parameters, such as Hildebrand solubility parameters and group contribution concepts. But Hildebrand solubility parameters are only useful for qualitative descriptions of chromatographic behavior; therefore the group contribution concept is often used, since it was developed to predict activity coefficients in nonelectrolyte liquid mixtures (Petrovic et al., 1985). This concept is the basis for the UNIFAC model for chromatographic retention, which combines a model based on extension of quasi-chemical theory of liquid mixtures (UNIQUAC) with the concept of functional group solubility. In this method solute activity coefficients in the mobile and stationary phases are calculated via structural and binary parameters characterizing the mutual interaction energy of the

functional groups in the system. The solute activity coefficient is the product of a combinatorial and residual contribution. The combinatorial contribution is dependent on the size and shape of the molecules in the system; the residual depends on the interaction energy of functional group pairs, as well as the fraction of the surfaces on these groups which are available for mutual interactions (Petrovic et al., 1985).

Assuming infinite dilution, the relationship between the capacity factor of a solute i and its activity coefficient (f_i) in the mobile and stationary phases can be written as

$$\ln k_i' = \ln K_i + \ln (V_S/V_m)$$

therefore $\ln k_i' = \ln f_{im} - \ln f_{is} + \ln (V_S/V_m)$. Petrovic et al. (1985) assume that f_{is} and V_S/V_m are constants; therefore $\ln k_i'$ and $\ln f_{im}$ are linearly related with a slope of one. If the activity coefficients of the solute i in the chromatographic system and V_S/V_m are known, retention can then be predicted. Petrovic et al. (1985) calculated infinite dilution activity coefficients of solutes in the mobile phase from experimental gas-liquid chromatographic data and then correlated them with their RP retention values to see how well the UNIFAC method could predict chromatographic behavior. They assumed that V_S/V_m would be the same for any octadecyl RP column at any methanol/water mobile phase composition (an assumption that will be thoroughly disputed in Chapter IV of this tome) and that

In f_{is} was zero (since stationary phase interactions were assumed to be very weak and nonselective). They found that the predicted values of k' were at best a rough estimate of actual chromatographic retention and concluded that therefore the solute interaction with the stationary phase could not be ignored in the prediction of RP retention. However they found the UNIFAC method to be useful for predicting changes in relative solute retention with varying mobile phase composition, since the solute activity coefficients in the mobile phase could be accurately calculated using UNIFAC parameters (Petrovic et al., 1985).

Hydrophobicity is the physical descriptor which has most accurately been used to predict RPLC retention. Hydrophobic effects between solutes and hydroorganic mobile phases were described earlier in this chapter. Solute hydrophobicity is usually described in terms of the π scale developed by Hansch and Leo (1979). By evaluation of solute partition coefficients between *n*-octanol and water (P) they were able to establish substituent hydrophobicity parameters. The logarithm of the partition coefficient is determined for both a compound containing the substituent group and the parent compound; the difference in these two $\log P$ values is π , the hydrophobicity parameter for the substituent (Melander and Horvath, 1980). Jinno (1982) and Jinno and Kawasaki (1984a and 1984c) use the descriptors π , HA and HD, where HA is the number of electron acceptor groups and HD is the number of electron donor groups, to

correlate with RPLC retention for substituted benzenes (excluding phenol). They found a very good correlation between $\ln k'$ and π , and poor correlations between $\ln k'$ and molecular connectivity, correlation factor (number of double bonds plus number of primary and secondary carbons minus 0.5 for nonaromatic rings) and van der Waals volume and surface area. They interpret this to mean that the size and shape of these molecules were not dominant for controlling their retention. However, when the size and shape of the solute molecules are a dominant retention force such as for PAHs and hydroaromatics, the correlation factor (F) has been found to correlate quite well with $\log k'$ (Hurtubise et al., 1982). If a linear combination of HA, HD and π descriptors was used, they found an even better correlation between predicted and experimental k' values. For phenol solutes, hydrophobicity alone was not an adequate retention descriptor so they added Hammett's acidity parameters (σ) to account for the strong hydrogen bonding ability of the phenols (Jinno and Kawasaki, 1984a and 1984c). Linear combinations of π and σ for the phenol solutes resulted in excellent correlations with retention behavior. However, for both sets of solutes the parameters had to be multiplied by certain proportionality constants in order to correctly predict retention and these constants were different not only for different organic modifiers in the mobile phase but also for each volume composition. This requires that at every different mobile

phase composition a least squares fitting for a large data set must take place in order to determine these proportionality constants, requiring a tremendous amount of data both in terms of π (and possibly σ) values for each solute and in terms of retention values for these solutes at each mobile phase composition. Jinno and Kawasaki (1984a and 1984c) also have not shown that this model is applicable for larger more complex molecules.

Funasaki et al. (1986) examined retention behavior for alcohols and ethers with positional and geometric isomers and examined the correlations between the solute's $\log k'$ and molecular cavity surface area (S), the logarithm of the aqueous solubility ($\log C_w$) and the logarithm of the octanol-water partition coefficient ($\log P$). As previously mentioned in this chapter, it was found that S and k' were very well correlated, even in the case of conformational isomers. This is because S for a molecule in water is defined as the area of the surface traced out by the center of a water molecule rolling over the van der Waals surface of the solute molecule (Funasaki et al., 1986). However S is very difficult to accurately calculate, requiring the construction of solute molecular models for rigorous work, as well as detailed knowledge of the molecular conformation of the molecule of interest. If S is calculated from group surface areas, an easier but less rigorous approach, the molecular surface area is usually overestimated for very crowded molecules. Using S to predict retention does have

one very strong merit--since the slope of a $\log k'$ versus S plot is related to interfacial tension, the dependence of $\log k'$ on the organic modifier content of the mobile phase can be predicted (Funasaki et al., 1986).

Funasaki et al. (1986) found the correlation between $\log k'$ and the logarithm of solute aqueous solubility, C_w , to be rough at best. The main drawbacks to this method are that the extent of correlation will depend on whether C_w was measured for the compound in the gas, liquid or solid state, that some compounds are infinitely water soluble and that the correlation is particularly poor for branched solutes. In contrast, they found that the correlation between the logarithm of the solute octanol-water partition coefficient ($\log P$) and $\log k'$ was particularly good for the solutes examined (alcohols and ethers) in methanol/water mobile phases. Braumann (1986) reports that other workers have found good correlations between $\log k'$ and $\log P$ for a variety of compounds including PAHs, alkylbenzenes, substituted benzenes, pesticides, phenols and barbiturates; again correlations were much better with methanolic mobile phases than with those containing acetonitrile, due to methanol's hydrogen bonding properties. One drawback to this method is that octanol-water partition coefficients are measured via shake-flask methods, which are very time consuming. However, P values are tabulated for many compounds and additivity rules using Hansch's π parameters may be used to estimate P for other compounds although

estimated P values do not in general correlate as well with retention as measured ones (Braumann, 1986; Funasaki et al., 1986). This approach is very much like that of Jinno and Kawasaki (1984a and 1984c) previously discussed except that Jinno and Kawasaki examined substituent group hydrophobicity (π parameters) in relation to retention whereas Funasaki et al. (1986) examined its relation with overall molecular hydrophobicity ($\log P$).

Funasaki et al. (1986) also examined the effect of temperature and mobile phase composition on the degree of correlation between $\log k'$ and $\log P$. Not surprisingly, they found that they were better correlated when both were measured at the same temperature than when they were measured at different temperatures. They also found that the $\log k'$ value estimated from extrapolation to zero percent methanol (i.e. totally aqueous) mobile phase gave a better correlation with $\log P$ than those obtained with hydroorganic mobile phases. As in all other cases previously discussed, the extent of correlation will be dependent upon the test solutes, the chromatographic column and the experimental conditions used (Funasaki et al., 1986).

In summary, most of the retention indices based on solute descriptors tend to accurately predict retention for certain small sets of similar compounds; they are by no means universal. Additionally, some of them require extensive calculations and/or experimental data in order to

predict retention. At present, none of these solute descriptor index systems is adequate for reliable prediction of RPLC retention.

Empirical Prediction of Retention

Jandera and coworkers (Colin et al., 1983a; Jandera, 1986; Jandera et al., 1982; Jandera and Spacek, 1986) have developed an empirical model to predict absolute or relative retention. They assumed that the stationary phase contribution to retention is very small compared to that of the mobile phase and that nonpolar interactions between the solute, stationary phase and mobile phase cancel each other. If this is true, the energy of transfer of the solute from the mobile to the stationary phase will depend on the interaction energy (energy of cohesion) between mobile phase molecules and the interaction energy between the mobile phase and solute. They defined an interaction index, determined from retention data in hydroorganic mobile phase systems, which describes polar interactions between solute molecules and the mobile phase components.

The interaction index for a solute (I_X) can be determined if the volume of interaction (V_X) for the solute and the column phase ratio (V_S/V_M) are known, since in this model,

$$\log (k_X'/V_X) - \log ((V_S/V_M)/V_X) = A - BI_X \quad (1)$$

where A and B are constants which depend on the stationary and mobile phases used. Jandera et al. (1982) plotted $(\log k_X' - \log (V_S/V_M))/V_X$ versus solute polarity (based on

Snyder's polarity index) to determine the value of I_X for "standard" solutes with different functional groups, and then found the average value of these I_X 's for a given solute in many different mobile and stationary phases. They then assumed that I_X will be constant for compounds which undergo the same type of interactions with the mobile phase as the "standard" solute does. Therefore if V_X for a similar compound is known, its retention can be predicted from equation 1, since A, B and I_X are already known from the standard data (Jandera et al., 1982).

Although this model is useful in a practical sense since it is based on empirical considerations, it cannot be completely justified in a theoretical sense. Although the solute experiences stronger interactions with the mobile phase than with the stationary phase, Jandera et al. (1982) do not prove that stationary phase interactions can be completely ignored. They also state that the standard compounds must be chosen "correctly" or else the predictive power of the model fails; "correct" solutes will be those with little or no specific interactions (such as hydrogen bonding) between the solutes and mobile phase components. This severely limits the types of compounds whose retention can be predicted from this model. Finally, at present the model cannot give very accurate (about 5 to 20% accuracy) retention predictions (Jandera et al., 1982).

Theories of Retention in RPLC

Solvophobic Theory

In order to truly understand the retention process in RPLC and thereby be able to predict solute retention, the retention process must be examined at the molecular level. At present there are two main schools of thought on the retention mechanism of RPLC at the molecular level. The solvophobic theory espoused by Melander and Horvath (1980) states that RP retention comes about from solute binding onto the stationary phase from the mobile phase and is mainly due to hydrophobic interactions between the solute and the mobile phase. Other workers have utilized statistical mechanical analysis based on mean field lattice theory to show that RPLC solute retention is due to solute partitioning from the mobile phase into the bonded stationary phase chains (Dill, 1987a and 1987b; Marqusee and Dill, 1986; Martire and Boehm, 1983). The main tenets of both of these proposed theories will be outlined below.

Melander and Horvath's (1980) solvophobic theory assumes that the mobile phase plays the dominant role in the RPLC retention process. This is because the stationary phase is nonpolar; therefore the only attractive forces occurring between the stationary phase and a nonpolar solute will be van der Waals forces, which are weak and nonspecific. They attribute the interactions between the solute and the mobile phase to a type of hydrophobic effect, which was discussed earlier in this chapter. In the

specialized RPLC environment, they have adopted a variation of this effect, termed the "solvophobic" theory, since the hydrophobic theory assumes a totally aqueous environment and RPLC mobile phases are generally a mixture of aqueous and organic components. Solvophobic theory is based on a theory of solvent effects on chemical equilibria developed by Sinanoglu (1968). The theory states that chromatographic retention is based on the free energy change as the solute is transferred from a hypothetical gas phase at atmospheric pressure to the mobile phase. The energy involved in this process is calculated in two steps. In the first, a cavity of the proper shape and size for the solute molecule is formed in the solvent. In the second, the solute enters the cavity and interacts with the surrounding solvent molecules via van der Waals and electrostatic interactions (Melander and Horvath, 1980).

The free energy change accompanying the mobile phase cavity formation comes about from the fact that the solvent surface area will increase by the molecular surface area of the solute (Melander and Horvath, 1980). Therefore the mobile phase free energy will increase by an amount proportional to the solvent surface tension and the increase in area. The change in free energy due to the interaction of the solute with the surrounding solvent molecules will be due to chemical and entropic effects. The chemical effects are van der Waals interactions and electrostatic effects. The van der Waals interaction energies are a function of the

polarizability and ionization potentials of the solute and solvent species. Electrostatic interactions consider both dipole and ionic effects; dipole effects are calculated from the solute dipole moment, polarizability and molecular radius as well as from the dielectric constant of the solvent. Ionic effects are calculated from conventional electrostatic theories such as Debye-Huckel treatments. The entropic term is a measure of solute "free volume," which is the volume that the molecule encounters before colliding with another molecule. The "free volume" is assumed to be proportional to the solute molar volume (Melander and Horvath, 1980).

Although the solvophobic theory outlined above is pertinent to a liquid-liquid system, the bonded stationary phase has not been considered in this process. Melander and Horvath (1980) regard the change in free energy for retention to be a combination of the mobile phase effects just described and a small contribution from the adsorption of the solute onto the stationary phase surface. This adsorption is viewed as a reversible reaction between the solute and stationary phase to form an associated complex. The free energy of adsorption is quantified as the van der Waals interaction energy between the solute and stationary phase in the absence of solvent molecules. Although Melander and Horvath (1980) mention that an entropic term should be introduced to account for the restricted translational freedom of the bonded chains at the silica

surface, they ignore this effect because they feel its contribution is negligible. In summary, in the solvophobic theory of RPLC retention, retention is mainly dependent upon the free energy of creation of a solute sized cavity in the mobile phase; stationary phase effects are considered to be weak and therefore rather negligible.

Partitioning Theory

One of the most severe drawbacks to Melander and Horvath's (1980) solvophobic theory is that it is based on a one phase model--that of the mobile phase. But RPLC involves two phases, the stationary and mobile phases; therefore a one phase model is not completely applicable for such a system. Melander and Horvath (1980) view the retention process as if there is no true transfer of the solute from the mobile phase to the stationary phase; the solute is merely associated with the stationary phase through weak adsorptive effects. Melander and Horvath (1980) also account for the stationary and mobile phase interactions by viewing them as bulk phases with homogeneous properties throughout. In reality, the stationary phase/mobile phase boundary is a highly heterogeneous area consisting of the core silica particles, the alkyl chains bonded to the silica surface, residual silanol groups remaining on the silica surface and the mobile phase solvating these silanols and the bonded chains. In such a heterogeneous system, it is highly unlikely that bulk phase thermodynamic considerations based on ideal solution behavior are applicable (Marqusee and Dill, 1986).

In their adsorption model, Melander and Horvath (1980) use very simple descriptions of the stationary phase surface which contain two gross oversimplifications. They imply first of all that the bonded chains are rigid rods containing no internal degrees of freedom. But at the temperatures commonly used in RPLC, the bonded alkyl chains are quite disordered (Dill, 1987a). Melander and Horvath (1980) also use stationary phase models wherein the bonded alkyl chains are fully exposed to the mobile phase. However, in a hydroorganic mobile phase system the chains cannot be fully exposed to such a highly aqueous environment; such a configuration would be prohibitively expensive in free energy terms (Dill, 1987a).

Dill (1987a) has proposed an alternative model of the RPLC stationary phase surface which regards the grafted phase as an organized "interphase" similar to those found in surfactant aggregates such as monolayers, bilayers, micelles and microemulsions (Marqusee and Dill, 1986). Interphases are composed of alkyl chains that have one end anchored at an interface; their thickness is on the order of a few molecular dimensions. The anchored chain density (i.e. the number of chains anchored per unit silica surface area) is sufficiently high as to cause severe configurational constraints. Two important properties distinguish this system as an interphase: its surface area/volume ratio is high and its properties vary with the distance from the anchored end. The relationship between orientational order

and distance from the silica surface is termed a "disorder gradient"; the bonded chains have much greater orientational order at the anchored ends and this order decreases with the distance from the attached end. This is in contrast to bulk matter phases in which by definition properties are invariant with spatial position (Dill, 1987a).

In Dill's (1987a) retention theory, the nature of the retention process is dependent upon the nature of the molecular organization within the interphase. There are three factors which determine this organization: the first is those constraints imposed by the surface density and chain lengths of the alkyl groups bonded to the surface and by the surface's geometry; the second requirement is that in highly aqueous mobile phases the interphase region must largely exclude the solvent due to hydrophobic effects; the interphase volume will be filled by chain segments and solute molecules. The final requirement is that the chains adopt as much disorder as is consistent with the other two constraints in order to conform to the second law of thermodynamics. This approach allows the consideration of any possible geometry of the silica surface onto which the chains are bonded. Dill (1987a) assumes that the silica surface is approximately planar; in terms of molecular dimensions this should be a good approximation for chromatographic silicas, which commonly have pore diameters of 60 to 100 Angstroms or more; the effects of curvature will be small unless the radii of curvature are a few molecular chain lengths or less (Marqusee and Dill, 1986).

Dill (1987a) considers partition and adsorption separately as alternative RPLC retention mechanisms. In both cases a lattice interphase model is used for the bonded phase surface and statistical thermodynamic calculations are used to predict solute retention in the system (Dill, 1987a; Marqusee and Dill, 1986; Martire and Boehm, 1983). Dill (1987a) predicts the equilibrium partition coefficient for a solute from the mobile phase to the stationary phase from the chemical potentials of the solute in the mobile phase system and in the bonded chain interphase. These calculations include the entropy of mixing of the solute and solvent in the mobile phase, the decrease in configurational entropy of the bonded chains when the solute is inserted within the interphase and the total contact free energy of the system, which will be due to intermolecular interactions of the molecules with their neighbors. After careful consideration of both the partitioning and adsorption retention mechanisms in conjunction with his interphase model and available experimental evidence, Dill (1987a) concludes that the principal retention mechanism in RPLC is partitioning due to two lines of evidence. Partitioning will be affected by the surface density of the bonded alkyl chains; adsorption will not. Therefore if partitioning is dominant, after a certain critical bonding density, solute retention should decrease with increasing alkyl chain surface density. It has been observed that there is less solute retention in bonded alkyl phase stationary phase

systems than in the corresponding bulk alkane systems (Colin et al., 1983b). Although Melander and Horvath (1980) have interpreted this as favoring an adsorption mechanism, Dill (1987a) interprets this in terms of partial chain ordering in the stationary phase, leading to less retention than in the completely disordered bulk alkane. This is supported by the work of Lochmuller and Wilder (1979) since solute methylene selectivities should be unaffected by the molecular organization of the interphase (Dill, 1987a).

The other line of evidence is that $\ln k'$ for congeneric sets of molecules can be linearly correlated with $\ln P$ (octanol-water partition coefficient) with a slope of one. A slope of one is expected for the partitioning mechanism, since all of the solute surface area would be available for partitioning within the interphase. The slope for the adsorption mechanism is expected to be considerably less (about 1/6) because only a small fraction of the solute surface area would contact the hydrocarbon chains, giving a smaller driving force for retention (Dill, 1987a). Experimental evidence has resulted in linear plots of $\ln k'$ versus $\ln P$ with a slope of one (Melander and Horvath, 1980).

Based on the partitioning mechanism of retention, Dill (1987b) predicts that at low surface densities (less than 2.7 micromoles of bonded alkyl chains per square meter of silica surface) nonpolar solute retention will increase linearly with increasing surface density. At these low

densities, chain configurational constraints are very small and interphase chain packing will have no effect on solute retention; solute retention will increase as the volume of chains increases since there will be more alkyl phase for the solutes to partition into. At a chain density of zero (bare silica) the nonpolar solute will be unretained (Dill, 1987b).

Once a critical bonding density (predicted to be about $2.7 \mu\text{mol}/\text{m}^2$) is reached, the bonding density is high enough for severe configurational constraints to result. In the Dill (1987a) retention model, the free energy involved in retention is determined by the differences in the free energy between the creation of a solute sized cavity in the interphase region and the destruction of a solute sized cavity in the mobile phase. As alkyl bonding density is increased past the critical bonding density, the chain packing constraints become more and more severe, requiring larger amounts of energy to create a solute cavity in the interphase structure. Thus in the high density region solute partitioning is predicted to decrease with increasing alkyl bonding density due to the increasingly prohibitive expenditure necessary for interphase cavity creation to accomodate the solute. Dill (1987a) predicts that at $8.1 \mu\text{mol}/\text{m}^2$, the maximum achievable bonding density if every surface hydroxyl group on the silica is derivatized with an alkyl ligand, solute retention would be zero and the solute would be completely excluded from the interphase chain packing structure.

Some researchers have examined solute retention as a function of increasing alkyl chain length of the bonded ligands. Colin et al. (1983b) and Jinno and Kawasaki (1984c) found that $\log k'$ increased with increasing bonded chain length, while Melander and Horvath (1980) have stated that more or less contradictory results have appeared in the literature on the influence of chain length. Spacek et al. (1980) and Berendsen and de Galan (1978b) have also noted a general trend of increased retention with increasing chain length of the bonded ligand, but their results were less conclusive than those of Jinno and Kawasaki (1984c). It is not clear from any of these studies whether this trend is due to the actual increased partitioning of the solute into the longer alkyl chains or whether this trend is an artifact of the retention parameter measured. This is because in all of these studies, it is the capacity factor, k' , that is used to quantitate retention. But $k' = K(V_S/V_M)$ where K is the chromatographic partition coefficient and V_S/V_M is the volume phase ratio of the stationary and mobile phases. It is obvious that as the chain length of the bonded alkyl ligand is increased, a corresponding increase in V_S will occur, as pointed out by Colin et al. (1983b). Therefore it is unclear as to whether solute retention increased because of increasing partition coefficient or merely because of the phase ratio increase. In order to reliably determine the cause of changes in actual solute retention as stationary phase parameters are changed, the changes in the solute partition coefficient must be examined.

In this study, we examined the effects of octadecyl alkyl chain bonding density on both retention and selectivity of small nonpolar solutes. We were particularly interested in experimental verification of the molecular mechanism of RPLC retention proposed by Dill (1987a and 1987b). Although Sander and Wise (1984a and 1984b; Wise and Sander, 1985) and Wise and May (1983) have extensively examined the effect of alkyl bonding density on retention and selectivity for PAHs, they have mainly examined polymeric stationary phases, which are not as well structurally characterized as the monomeric stationary phases used in our study. Another problem with their work is that they examined capacity factor (k') behavior in their retention studies, which fails to account for phase ratio changes. Additionally, PAHs are not ideal solutes for such a study, since their large sizes and unusual shapes are not typical of most chromatographic solutes.

In order to determine the effect of interphase chain packing on solute partitioning, the behavior of the chromatographic partition coefficient was examined as a function of octadecyl bonding density. Chromatographic selectivity was also studied as a function of bonding density for solutes of different sizes and shapes. Novel synthetic methods utilizing ultrasound as a reaction driving force were devised to obtain stationary phases with high bonding densities. In this manner, we were able to see if Dill's (1987a and 1987b) proposed RPLC retention mechanism

was verified by actual chromatographic behavior. These experiments were carried out using both silica and controlled pore glass substrates in order to compare the two materials as stationary phase supports.

CHAPTER II SYNTHESES OF SILICA-BASED RP STATIONARY PHASES

Experimental Considerations in the Synthesis of RP Stationary Phases

Reversed phase bonded silicas are the most popular packings used in high performance liquid chromatography (HPLC). Although the role of the mobile phase in chromatographic retention and selectivity has been extensively studied, that of the stationary phase has only come under intense scrutiny recently and as a result the effects of the stationary phase on these chromatographic properties is not yet fully understood. One reason for this dearth of knowledge is the lack of precise and reliable methods for determining bonded phase characteristics such as the density, homogeneity and topographical distribution of the bonded alkyl ligands and the residual hydroxyl groups on the support surface. These properties are a direct consequence of the bulk silica medium and the reagent and reaction conditions for the silanization process (Kinkel and Unger, 1984). In order to obtain reversed phase packings with reproducible surface characteristics, the silanization reaction conditions must be painstakingly controlled.

In the preparation of reversed phase packings, one objective is the modification of as many surface hydroxyl

groups on the silica as possible, especially the highly acidic isolated silanols. These residual isolated silanol groups have been shown to be the main cause of tailing of chromatographic peaks for basic compounds, of mechanical instability of the packing, and of low sample capacity of the column (Kohler et al., 1986; Kohler and Kirkland, 1987). Di- or trireactive alkylsilanes had previously found favor over monoreactive silanes because of their greater reactivity and the possibility of reacting simultaneously with two or three hydroxyl groups. However, any unreacted sites on the bonded functional groups will be hydrolyzed upon contact with water (i.e. from the mobile phase), forming additional undesirable silanol groups (Berendsen and de Galan, 1978b; Snyder and Kirkland, 1979). Di- and trireactive silane reagents also often result in nonreproducible stationary phases since the degree of polymerization is highly dependent on the residual water content of the silica and the reagents used in the bonding reaction (Snyder and Kirkland, 1979). Another drawback of polymeric stationary phases is their lower chromatographic efficiency, which results from poor solute mass transfer in these relatively thick stationary phases. Therefore many investigators now advocate the use of monofunctional silanes for the silica derivatization reaction, since this results in a reproducible and well defined chemically bonded phase. Additionally, monomeric stationary phases generally exhibit superior column performance to polymeric phases due to their

faster solute mass transfer kinetics (Cooke and Olsen, 1980). For octadecyldimethylchlorosilane, the most commonly used monoreactive silane, the resulting bonding reaction is depicted in Figure 2-1.

Kinkel and Unger (1984) have studied the roles of the solvent and the base in these monofunctional bonding reactions and have found their choice to be crucial. When alkylhalosilanes are reacted with silica, a base is added to serve as the acid-acceptor catalyst, binding the haloacid formed during the reaction and driving the equilibrium to the product side. In addition, the base favorably affects the kinetics of the silanization reaction. Mechanistic studies of these types of reactions (Corriu and Guerin, 1980) have shown that two molecules of base attack one molecule of silane, activating the Si-X bond such that a reactive intermediate and a hydrohalide are formed. Formation of this reactive intermediate greatly increases the kinetics of the bonding reaction; indeed, the addition of the acid-acceptor catalyst results in approximately 90% of the total conversion taking place within the first hour of the reaction. In their study, Kinkel and Unger (1984) found that the two most effective acid-acceptor catalysts for organohalosilanes were imidazole and 2,6-lutidine.

The reaction solvent must also be carefully chosen. The solvent can interact specifically with the silane, the base and the surface silanol groups on the silica. When the solvent interacts with a silanol group, there is a

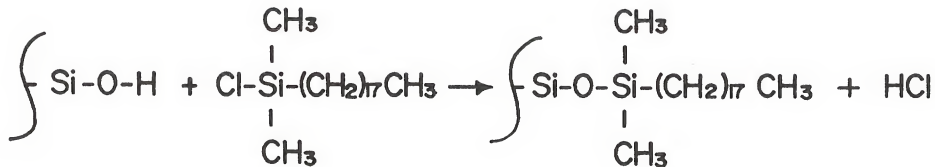


Figure 2-1. Bonding reaction for monomeric octadecyl reversed phase packings.

considerable effect on the strength of the bond between the silicon and oxygen atoms. Solvents which have both a pronounced Lewis acid and Lewis base character cause the Si-O bond strength to be weakened and facilitate the bonding reaction. The solvent can also activate the silicon atom of the organohalosilane by forming a pentacoordinated intermediate through nucleophilic attack. The resultant bond lengthening causes nucleophilic activation to occur, favoring attack by a second nucleophile (such as the base). The solvent may influence the base as well, as it is known that in aprotic polar solvents the nucleophilic character of reactants is more pronounced. All of these considerations may have a synergistic relationship as well. Based on their experimental work with organohalosilanes, Kinkel and Unger (1984) found that methylene chloride and N,N-dimethylformamide were the most effective solvents for the bonding reaction.

Many organic reactions have been shown to be enhanced by ultrasound (Boudjouk, 1986; Bremner, 1986; Clough et al., 1986; Han and Boudjouk, 1982 and 1983; Suslick, 1986). Boudjouk and Han (1981) have shown that in the presence of ultrasonic waves both alkyl and aryl chlorosilanes could be coupled over lithium wire; without ultrasonification, this reaction occurred to no appreciable extent. Reactions at solid-liquid interfaces are also particularly enhanced by ultrasound (Bremner, 1986; Suslick, 1986). It is then reasonable to assume that reversed phase bonding reactions

might be facilitated under ultrasonification. The use of ultrasound has two distinct advantages over traditional reflux methods. The ultrasonic waves serve as a driving force which is controlled independently of temperature, allowing reaction temperatures to be varied over any desired range. Secondly, the power of the ultrasonic driving force can be varied by using a variable power ultrasonic probe.

We have investigated the effect of ultrasound on the silane bonding reaction, including the effects of subambient and superambient temperatures on the ultrasonic reaction. In addition to these investigations, a novel base, 4-dimethylaminopyridine, was utilized as the acid-acceptor catalyst, in hopes that it might prove superior to 2,6-lutidine.

Experimental Procedure

Reagents

All of the organic solvents used were supplied by Fisher Scientific (Fairlawn, NJ). Water was deionized, passed through a Barnstead Nanopure (Boston, MA) purification system, irradiated in a Photronix Model 816 HPLC reservoir with a UV source (Photronix Corp., Medway, MA) for at least 48 hours, and filtered through a 0.45 μm Nylon 66 membrane (Rainin, Woburn, MA). The methanol used was HPLC grade; the chloroform, methylene chloride, and diethyl ether were reagent grade. Methylene chloride was dried by stirring over phosphorus pentoxide (Fisher Scientific) for 24 hours, followed by distillation under a dry nitrogen atmosphere.

Dimethyloctadecylchlorosilane, n-octyldimethylchlorosilane and trimethylchlorosilane (99.9%) were used as received from Petrarch Systems (Bristol, PA). The 2,6-lutidine (Sigma Chemical Co., St. Louis, MO) was stirred for 24 hours over barium oxide (Fisher Scientific) prior to distillation under dry nitrogen atmosphere; 4-dimethylaminopyridine (4-DMAP; Nepera Inc., Harriman, NY) was oven dried at 80 °C for 24 hours before use.

The chromatographic silica was from a single lot of Davisil (W. R. Grace, Baltimore, MD) synthetic amorphous silica, grade 641LCOX1823. The silica had an average pore diameter of 147 Angstroms, an absolute surface area (S_{BET} , as measured by BET analysis) of 300 m²/g, a particle size range of 20-30 μ m with an 80% distribution of 23 ± 10 μ m and a nitrogen pore volume of 1.10 cm³/g (Grace, 1984). As recommended by Snyder and Kirkland (1979) the silica was acid leached in 0.1 M nitric acid at 90 °C for 24 hours in order to fully hydroxylate the surface and to remove any metal contaminants remaining from the manufacturing process. The silica was then washed thoroughly with water until all traces of the nitric acid had been removed and dried under vacuum at 240 °C for 24 hours prior to use in order to remove physically adsorbed water from the surface (Unger, 1979). Scanning electron micrographs of the acid-leached silica (Figures 2-2 and 2-3) show that this type of chromatographic silica exhibits an irregular shape as well as an irregular surface. This surface irregularity is reflected in the silica's high surface area.

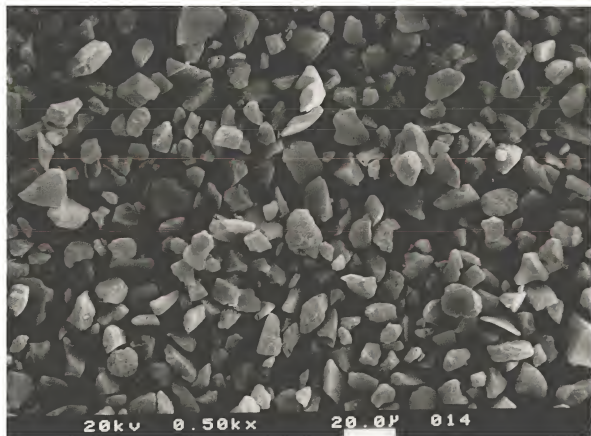


Figure 2-2. Scanning electron micrograph of acid-leached Davisil silica; 500X magnification.

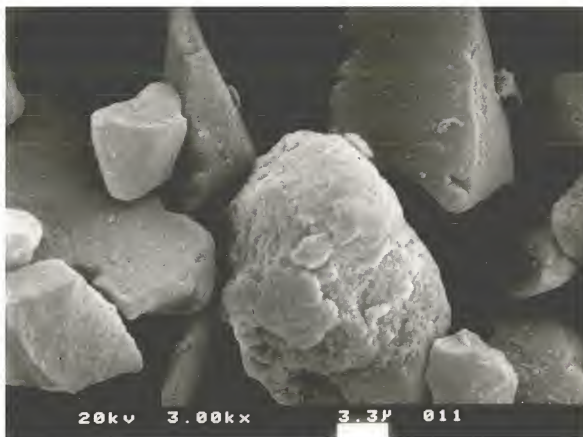


Figure 2-3. Scanning electron micrograph of acid-leached Davisil silica; 3000X magnification.

Silane Bonding Reaction

It is essential that the silane bonding reaction be carried out under scrupulously dry conditions in order to prevent the water-initiated dimerization of the silane reagent. Glassware used in the derivatization reaction was presilanized by etching the surface with a 10% (v/v) hydrofluoric acid (Fisher Scientific) solution, drying, and then soaking the glassware for an hour in a 5% (v/v) trimethylchlorosilane in chloroform solution. Immediately prior to use, the glassware was oven dried at 125 °C for at least 4 hours in order to remove trace moisture and allowed to cool in a dry box under nitrogen atmosphere. The reagents were mixed together in the dry box and the reaction flasks kept under dry nitrogen atmosphere at all times. Based on Kinkel and Unger's (1984) estimation of a maximum of five micromoles of reactive hydroxyl sites per square meter of silica surface, a twofold excess of the silane reagent was added to achieve exhaustive derivatization of the silica surface. A fourfold excess of the base (2,6-lutidine or 4-DMAP) was added both to serve as an acid-acceptor catalyst for the HCl produced in the reaction and to act as a reactive intermediate at the silica-solution interface. Dry methylene chloride was used as the reaction solvent, using a ratio of 10 ml of methylene chloride per gram of base silica.

The reaction flasks were sonicated by immersion to the flask neck in an ultrasonic cleaning bath (Bransonic model

B-2200R-1, Branson Cleaning Equipment Co., Shelton, CT) with a power rating of 100 W and a frequency of 55 kHz. Stirring of the reagents within the flasks was accomplished by rotating a magnetic bar submerged in the bath adjacent to the reaction flask, resulting in the corresponding rotation of a magnetic stirring bar within the flask. Temperature control of the ultrasonic bath was accomplished by passing a thermostatted solution of ethylene glycol and water through coiled copper tubing lining the inner perimeter of the bath. The solution was thermostatted by an Endocal or Exacal water bath (Neslab Instruments, Portsmouth, NH). Refluxed reactions were carried out at 50 °C using an oil bath and magnetic stirrer. Control reactions were carried out by stirring the reaction mixture at room temperature.

Refluxed reactions utilizing n-octyldimethylchlorosilane as the reactive silane, 2,6-lutidine as the acid-acceptor catalyst and methylene chloride as the reaction solvent were carried out for reaction times of 24, 36 and 48 hours in order to ascertain whether the differences in reaction time would make a statistically significant difference in the reaction yield. Nine replicate reactions were performed for each reaction time. Student's t (t_{calc}) was calculated from the pooled standard deviation of percent carbon for all 27 reaction yields, the differences in the mean percent carbon at each reaction time, and the number of replicates at each time in order to determine if the mean yield for each reaction time was statistically different

from those at the other reaction times. In the comparison of the 24 hour reactions to the 36 hour reactions, t_{calc} was 0.984; for the 24 hour reaction time versus the 48 hour reaction time, t_{calc} was 0.148 and for the 36 hour reaction time versus the 48 hour reaction time, t_{calc} was 0.835. Since all of the calculated t values are less than the critical t value (t_{crit}) at the 80% confidence level ($t_{\text{crit},80\%} = 1.34$ for 16 degrees of freedom), there is no significant statistical difference in the reaction yields for reaction times of 24, 36 and 48 hours at the 80% confidence level (Peters et al., 1974). Therefore, reactions were carried out for 24 hours (as also recommended by Kinkel and Unger (1984)) unless otherwise noted.

Once the reaction time was complete, the product was washed in order to remove excess reagents. Each bonded phase product was washed three times with each solvent using the rinse sequence methylene chloride, methanol, 50/50 (v/v) methanol/water, methanol and diethyl ether. After the ether was allowed to evaporate from the product, the derivatized silica was dried in a vacuum oven at 125 °C for 16-24 hours. (Caution: It is imperative that the ether be completely evaporated from the product prior to drying the product in the vacuum oven in order to avoid a possible explosion.) Products were analyzed by in-house elemental analysis performed at least in duplicate for each sample. Reliability of the elemental analysis was confirmed by repeated submission of a standard packing material over a

two year period; for 66 measurements the resultant standard deviation was $\pm 0.20\%$ carbon.

Syntheses Utilizing Ultrasonic Waves

Use of Ultrasound as a Reaction Catalyst

The region of frequencies above 16 kHz is beyond the sensitivity of the human ear; it is therefore termed the ultrasound region. The first reported use of ultrasound in organic chemistry was in 1938, but it was not until the late 1970s that ultrasound was used to speed reactions in nonaqueous media; indeed the use of ultrasound as a reaction catalyst is still in its infancy (Boudjouk, 1986; Bremner, 1986). Ultrasonic radiation can be introduced to the reaction medium either by immersion of the reaction vessel into the liquid of a common laboratory ultrasonic cleaning bath or by introduction of an ultrasonic generating probe directly into the reaction medium.

Ultrasonic frequencies span the range of 20 kHz to 10 MHz, with associated acoustic wavelengths of 7.6 to 0.015 cm. Therefore sonochemistry cannot be accounted for in terms of direct coupling of the acoustic field with chemical species on a molecular level (Suslick, 1986). However, the effects of ultrasound can be attributed to three different phenomena. The variation of sonic pressure causes the rapid movement (oscillation) of fluids, subjecting them to compression and rarefaction. Negative pressure in the rarefaction region gives rise to cavitation, the formation and collapse of microbubbles. The violent implosion of

these microbubbles generates powerful shock waves with a considerable energy output (Boudjouk, 1986; Bremner, 1986; Suslick, 1986). Pressures in the kilobar range and temperatures of 2000-3000 °C have been estimated in the region of the collapsing bubble for time periods in the nanosecond range (Sehgal et al., 1980). The third contributing phenomenon is microstreaming, where a large amount of vibrational energy is put into small volumes with little heating (Bremner, 1986). The extremes of temperature and pressure generated by ultrasonic waves cause the generation of free radicals and ions, the dispersion of chemical layers and the promotion of intimate contact between reactants. Emulsification of immiscible liquids and enhanced mass transfer at solid-liquid interfaces are secondary effects of ultrasonification. All of these effects can contribute to the promotion of chemical reactions (Bremner, 1986; Suslick, 1986).

Effect of Ultrasound on the Bonding Reaction

In order to define the surface coverage of the bonded silica in an unambiguous and pertinent manner, the surface coverage should be expressed as the number of silane molecules attached to the surface, usually as micromoles of bonded silane molecules per square meter of silica surface, taking into account the increase in weight of the silica after the bonding reaction. These surface coverages are calculated from the percentage of carbon as obtained from elemental analysis of the bonded phase (Berendsen and de

Galan, 1978b). This calculation is quite straightforward for monoreactive silanes and for monochlorosilanes (the most commonly used monoreactive reagents) can be expressed by

$$\alpha = \frac{(\%C) (10^6)}{(12.011) (n_c) (S) (100 - \frac{(\%C)}{(12.011)(n_c)} (M - 36.5))} \quad (1)$$

where α is the surface coverage ($\mu\text{moles/m}^2$); %C is grams carbon per 100 grams bonded silica, as obtained from elemental analysis; n_c is the number of carbon atoms per mole silane; M is the molecular weight of the silane; and S is the surface area of the native silica in m^2/g . Although the typical value for the average surface hydroxyl concentration of amorphous silica is $8 \mu\text{mol/m}^2$ (Cheng and McCown, 1985), in practice octadecyl bonded phase coverages are limited to about $3 \mu\text{mol/m}^2$ due to steric considerations (Berendsen et al. 1980a; Berendsen and de Galan, 1978a and 1978b; Cheng and McCown, 1985; Snyder and Kirkland, 1979).

Three sets of experiments were compared in order to determine the effect of ultrasound on the silica bonding reaction. In all cases dimethyloctadecylchlorosilane was the reactive silane, methylene chloride was the reaction solvent and 2,6-lutidine the acid-acceptor catalyst; all reaction mixtures were stirred during the reaction time period (24 hours). In the first set of experiments, the reaction mixture was stirred at ambient temperature (22.0°C); in the second set the reaction mixture was refluxed at 50.0°C . The third set of experiments was performed at 28.5°C , but the reaction vessels were immersed in an ultrasonic

cleaning bath. The refluxed stationary phases had an average bonding density (\pm one standard deviation over three trials) of $2.82 \pm 0.02 \mu\text{mol}/\text{m}^2$. The room temperature reaction resulted in a bonding density of $2.69 \mu\text{mol}/\text{m}^2$ with a range of $\pm 0.03 \mu\text{mol}/\text{m}^2$ over two trials; the ultrasound reaction gave a bonded phase (over two trials) with an average bonding density of $2.71 \pm 0.01 \mu\text{mol}/\text{m}^2$. The small bonding density difference between the stirred reaction at ambient temperature and the one at reflux temperature is not surprising as Lork et al. (1986) have shown that the bonding density increases slightly and in a linear fashion with increasing reaction temperature when monochlorosilanes are used as the silanizing reagent. These experimental results show that ultrasound is indeed a viable method for the bonded phase synthesis, giving results which are comparable to those obtained using traditional reflux techniques.

Effect of Subambient Temperature on the Ultrasound Reaction

Two sets of experiments were performed using ultrasound in conjunction with subambient reaction temperatures. In achieving high bonding densities one of the greatest obstacles is increasing steric hindrance at the silica surface as more and more bulky dimethyloctadecylsilyl groups are bonded to the surface. It is possible that at low temperatures the bonding density might be enhanced due to the increased order (decreased entropy) in a lower temperature system. It is here that the ultrasound reactions are most unique, as they allow the temperature of the

reaction to be controlled independently of the ultrasonic driving force. Additionally, low reaction temperatures have often been found to enhance reaction yields for ultrasonically catalysed chemical reactions. One explanation for this phenomenon is that low temperatures cause the vapor pressures of the reactants to be decreased, enabling increased efficiency of ultrasonically produced cavitation (Boudjouk, 1986; Bremner, 1986; Suslick, 1986). In order to overcome the slower kinetics expected at lower temperatures, reaction times were increased beyond the usual 24 hour time period.

In the first set of experiments, two reaction vessels were sonicated and stirred at 15.0 °C for 48 hours with a resultant average bonding density (\pm the range) of $2.74 \pm 0.00 \mu\text{mol}/\text{m}^2$. Since this result was little different from that at room temperature, it was decided to increase the reaction time as well as to decrease the reaction temperature. In this set of experiments, two reaction flasks were sonicated and stirred at 8.5 °C for 101 hours with a resultant average bonding density of $2.84 \pm 0.01 \mu\text{mol}/\text{m}^2$, a slightly higher value than for those ultrasound reactions run at higher temperatures. These preliminary results indicated that subambient temperatures could indeed enhance the ultrasonic silica bonding reaction.

The Use of 4-Dimethylaminopyridine as the Acid-Acceptor Catalyst

There are also advantages in the use of 4-dimethylaminopyridine (4-DMAP) as the acid-acceptor catalyst. The

presence of the dimethylamino group should serve to make this base better at forming a reactive intermediate with the silane than 2,6-lutidine. In addition it has a relatively high melting point (108-110 °C) which allows it to be oven-dried rather than necessitating distillation to remove adsorbed water. The odor of 4-DMAP is also quite mild in comparison to that of 2,6-lutidine.

The first set of experiments using 4-DMAP as the acid-acceptor catalyst was performed using methylene chloride as the solvent and dimethyloctadecylchlorosilane as the reactive silane. The reaction mixture was refluxed and stirred at 50.0 °C for 24 hours. The bonded phase product had an average bonding density (\pm the range for two trials) of $3.44 \pm 0.02 \mu\text{mol}/\text{m}^2$, much higher than that achieved in our previous syntheses using 2,6-lutidine ($2.82 \pm 0.02 \mu\text{mol}/\text{m}^2$). This bonding density is also greater than that ($3.34 \mu\text{mol}/\text{m}^2$) achieved by Kinkel and Unger (1984) under reflux conditions using methylene chloride and 2,6-lutidine. This is especially significant because the silane in our experiments was used as received from a commercial source; Kinkel and Unger synthesized and then distilled their silane under reduced pressure in order to obtain a reactive silane of the utmost purity. Silane purity has been shown to be a very important factor in obtaining high bonding densities (Kinkel and Unger, 1984).

The second set of experiments using 4-DMAP as the acid-acceptor catalyst was run with the same reagents as

described above; the reaction mixture was immersed in the ultrasonic bath and stirred at a temperature of 31.0 °C for 24 hours. The average bonding density of the resultant bonded phases (\pm the range for two trials) was $3.35 \pm 0.05 \mu\text{mol}/\text{m}^2$, again much higher than that achieved under similar circumstances using 2,6-lutidine as the acid-acceptor catalyst ($2.71 \pm 0.01 \mu\text{mol}/\text{m}^2$).

A low temperature ultrasound reaction was then carried out under the same conditions as stated above, with a reaction temperature of 4.0 °C for a duration of 97 hours. For the two trials, an average bonding density of $3.24 \pm 0.01 \mu\text{mol}/\text{m}^2$ was obtained. A second set of low temperature ultrasound reactions was performed under analogous conditions with a reaction temperature of 3.0 °C for 144 hours. The bonded phase resulting from this experiment had a higher bonding density than achieved in any of our previous attempts; the average \pm the range for the set was $3.60 \pm 0.01 \mu\text{mol}/\text{m}^2$. To our knowledge, this bonding density is higher than any previously reported in the literature using dimethyloctadecylchlorosilane as the reactive silane (Berendsen et al., 1980a; Cheng and McCown, 1985; Kinkel and Unger, 1984).

To ensure that the high bonding density in this second low temperature experiment was a result of the ultrasonic driving force as well as the lengthy reaction time, two other reactions were carried out. In one, the reaction was performed exactly as above (at 3.0 °C for 144 hours with

stirring) except that the reaction flask was not sonicated. In the other, the reaction was stirred for 144 hours, but the reaction mixture was refluxed at 50.0 °C rather than sonicated. From duplicate elemental analyses of each of these two materials, the average bonding density (\pm the range) for the silica stirred (but not sonicated) at 3.0 °C for 144 hours was $3.48 \pm 0.00 \mu\text{mol}/\text{m}^2$; that for the silica refluxed and stirred at 50.0 °C for 144 hours was $3.44 \pm 0.03 \mu\text{mol}/\text{m}^2$. Since the absolute error in the elemental analysis is $\pm 0.20\%$ carbon, which corresponds to $\pm 0.03 \mu\text{mol}/\text{m}^2$ for the octadecyl packings, the differences in bonding density between these two materials and the silica which was sonicated at 3.0 °C for 144 hours ($3.60 \mu\text{mol}/\text{m}^2$) is both real and significant. Therefore it can be concluded that subambient ultrasound reactions are especially efficacious for synthesizing stationary phases with very high alkyl bonding densities.

In order to investigate the effect of superambient temperatures on the ultrasonic bonding reaction, two types of experiments were performed. In the first, the reaction was carried out by stirring with the same reagents as previously described for a reaction time of 24 hours and with the ultrasonic bath maintained at a temperature of 50.0 °C. For two trials, the average \pm the range was $3.34 \pm 0.04 \mu\text{mol}/\text{m}^2$, virtually identical to that achieved under ambient ultrasonic conditions ($3.35 \pm 0.05 \mu\text{mol}/\text{m}^2$). In the second experiment, the reagents were stirred and sonicated

at 31.0 °C for 1 hour and then refluxed and stirred at 50.0 °C for an additional 23 hours, in hopes that the preliminary sonication of the reagents would permit greater accessibility of the reactive silane to silanols located deep within the silica pores. The resulting bonding density (for two trials) of $3.42 \pm 0.03 \mu\text{mol}/\text{m}^2$ is comparable to that achieved under reflux conditions alone ($3.44 \pm 0.02 \mu\text{mol}/\text{m}^2$). These results indicate that silica bonding reactions performed in an ultrasonic bath are not affected by superambient temperatures; this is in contrast to those performed at subambient temperatures, which were found to give an increasing yield as the temperature was decreased.

Experiments were also carried out in the ultrasonic bath at 28.0 °C for 24 hours using trimethylchlorosilane (TMCS) as the reactive silane, 4-DMAP as the base and methylene chloride as the reaction solvent. TMCS is a much smaller molecule than the octadecyl silane and therefore should approximate the maximum bonding density obtainable in these reactions when steric hindrance is minimized. The average bonding density achieved in the two trials was $3.51 \pm 0.01 \mu\text{mol}/\text{m}^2$; the octadecyl bonding densities achieved in the above reactions show that the TMCS bonding density at ambient temperatures can be exceeded under subambient conditions even with bulky octadecyl reagents. The results of these 4-DMAP experiments, as summarized in Table 2-1, demonstrate that it is indeed a superior acid-acceptor catalyst to 2,6-lutidine for reversed phase bonding reactions.

Table 2-1. Comparison of silica octadecyl bonding densities using 4-DMAP and 2,6-lutidine as acid-acceptor catalysts.

Reaction Conditions	Temperature (°C)	Reaction Time (h)	C ₁₈ Bonding Density (μmol/m ²)	
			4-DMAP	2,6-lutidine
Refluxed	50.0	24	3.44	2.82
Ultrasound	28.0	24	-	2.71
Ultrasound	31.0	24	3.35	-
Ultrasound	8.5	101	-	2.84
Ultrasound	4.0	97	3.24	-
Ultrasound	3.0	144	3.60	-
Stirred Only	3.0	144	3.48	-
Refluxed	50.0	144	3.44	-

The use of ultrasound as a driving force for reversed phase syntheses has been shown to be a viable synthetic procedure. Ultrasonic syntheses performed at subambient temperatures have proven to be especially effective for the production of high alkyl bonding density stationary phases. The use of 4-dimethylaminopyridine as the acid-acceptor catalyst is recommended due to its ease of use and the resulting high bonding densities. The small ranges of the bonding density for duplicate syntheses show that reversed phase packings with reproducible bonding densities can be synthesized by these methods.

CHAPTER III SYNTHESES OF CONTROLLED PORE GLASS-BASED RP STATIONARY PHASES

Comparison of Controlled Pore Glass and Silica as Supports for RP Stationary Phases

The most commonly used column packing materials for reversed phase liquid chromatography (RPLC) are based on microparticulate silica. As has been described in Chapter II, this material is modified by chemically bonding alkyl chains of the desired length onto the silica surface. The use of such siliceous supports is widespread due to their high reactivity and relatively low cost. Silica-based RP bonded phases also exhibit good column stability within the pH range of 2.5 to 7.5 (Melander and Horvath, 1980).

However, such materials are not without problems. The surface of silica gel is very porous in nature and there is a wide distribution in the size of these pores. This can affect chromatographic selectivity by causing size exclusion effects. This broad pore size distribution is one of the contributors to the problem of inhomogeneous energies of transfer between the stationary and mobile phases for solutes in RPLC, leading to distorted peak shapes and decreasing chromatographic efficiency. The effects of pore size and structure have received much attention in size exclusion chromatography, but these parameters have garnered

little attention in reversed phase systems (Sander and Wise, 1984b). At present, there is no satisfactory explanation of the effect of pore size distribution on the properties of hydrocarbonaceous bonded phases; however differences in the pore structure of the support material may account for some of the differences observed in the RPLC behavior of various commercial bonded phases having the same alkyl chain length but different siliceous substrates (Melander and Horvath, 1980).

Controlled pore glass (CPG[®]) offers an ideal medium for investigating the effects of pore size and structure on RP retention and selectivity. CPG, which has mainly been used in size exclusion chromatography, consists of nearly pure quartz glass with pores of uniform size. In CPG, the pore diameter is the same at the surface as it is in the interior of the particle; 80% of the pores show a deviation of less than $\pm 10\%$ from the nominal pore diameter (Fluka). Chemical modification of the surface of CPG is accomplished by reacting surface silanol groups with the appropriate reactive silane, as has been described for silica in Chapter II. Although such reactions have been performed to prepare CPG RPLC bonded phases (Dawidowicz et al., 1983; Dawidowicz and Rayss, 1985; Rayss et al., 1983; Suprynowicz et al., 1978 and 1985) commercial bonded phases based on CPG are currently impractical due to its much greater expense compared to that of silica.

The differences in the pore structures of silica and CPG come about from the differences in their chemical compositions and in their manufacturing processes. The manufacture of chromatographic silica is described in detail by Unger (1979). The starting materials in the manufacture of porous silica are soluble silicates such as sodium silicates, silicon tetrachloride or tetraalkoxysilanes. By adjusting the pH of an aqueous solution of the starting material within a range of 8 to 9, silica sols are made. In the sol, polysilicic acids are formed by polycondensation and polymerization, growing into colloidal particles ranging from 1 to 100 nm. The sol consists of spherically shaped, nonporous and amorphous discrete silica particles. Unless stabilized, the discrete particles in the sol aggregate, mainly due to gelling. The particles become linked together to eventually form a three dimensional packing of silica particles that is a gelatinous mass called silica hydrogel. The hydrogel is washed and water is then removed by heating. This dehydration results in shrinkage from the partial collapse of the globular hydrogel structure; the resultant xerogel consists of hard porous grains. The silica particles are also cemented together by dissolution-deposition processes. The conversion of the hydrogel to the xerogel is the origin of the porosity of chromatographic silica. This porosity comes about from compaction of the dispersed silica in the hydrogel; the pore space is made up of the interparticle interstices and voids. This results in

a totally porous structure; moreover these pores are quite irregularly shaped. Factors which can be varied to control the final pore structure include changes in the sol and/or hydrogel pH, changes in the duration of the hydrogel ripening (via stabilization of the sol), variation of pH during washing of the hydrogel, and substitution of an organic liquid wash for the hydrogel rather than an aqueous one (Unger, 1979). The chemical composition of the final amorphous silica can be exemplified by the composition of the Davisil silica used in our experiments; it consists of 99.60% by weight of SiO_2 and 0.10% Na_2O with the remaining 0.30% made up of other metal oxides (Grace, 1984).

The procedure used to produce controlled pore glasses was first reported by Wolfgang Haller in 1965 (1965a and 1965b). The starting material consists of a Vycor type glass consisting of 7% Na_2O , 23% B_2O_3 and 70% SiO_2 . The glass is crushed, fractionated to the desired particle size distribution by sieving, and then heated at approximately 600 °C for the desired number of hours. This heating period causes fusion to take place within the glass, resulting in the formation of microheterogeneous regions in the continuous silica network. This alkali borate-rich microphase is then removed from the glass by a series of acidic and basic leachings, resulting in a finished material which is porous throughout its entire volume (Dawidowicz et al., 1983). The diameter of the pores is determined by the length and temperature of the heat treatment (Haller,

1965a). The chemical composition of controlled pore glass is also different from that of amorphous silica; the composition of the finished product is typically 96% by weight of SiO_2 , 3% B_2O_3 , less than 1% Na_2O , and a trace amount of other metal oxides (Electro-nucleonics Inc., 1987).

Experimental Procedure

Reagents

All of the reagents used in the preparation of the reversed phase CPG packings were as described in Chapter II, with the exception of CPG being used as the support material instead of silica. All CPG was manufactured by Electro-nucleonics Inc. (Fairfield, NJ). The CPG denoted as CPG-86 was from a single lot of CPG-10-75A (Fluka Chemical Corp.; Hauppauge, NY) and had a mean pore diameter of 86 Angstroms with a pore size distribution of $\pm 9.8\%$. The absolute surface area (S_{BET} , as measured by BET analysis) was $153.1 \text{ m}^2/\text{g}$; the particle size range was $37\text{--}74 \text{ }\mu\text{m}$ and the nitrogen pore volume was $0.48 \text{ cm}^3/\text{g}$. The CPG denoted as CPG-167 was from a single lot of PG-170-400 (Sigma Chemical Co., St. Louis, MO) with a mean pore diameter of 167 Angstroms and a pore size distribution of $\pm 9.6\%$. The absolute surface area was $161 \text{ m}^2/\text{g}$, the particle size distribution was $37\text{--}74 \text{ }\mu\text{m}$ and the nitrogen pore volume was $1.0 \text{ cm}^3/\text{g}$.

Bonded Phase Preparation

Both CPG's were acid leached, dried and reacted with the appropriate silane reagents for derivatization as

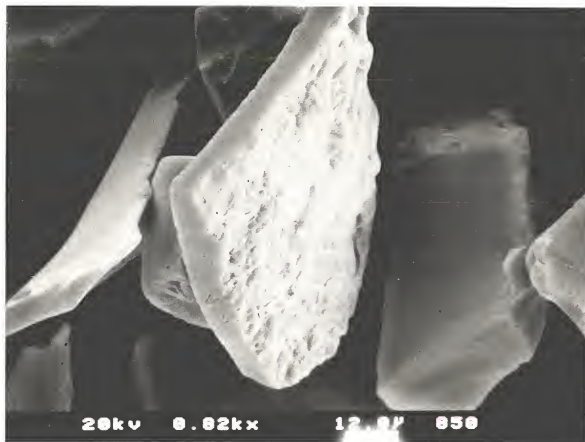


Figure 3-1. Scanning electron micrograph of acid-leached CPG-86; 820X magnification.

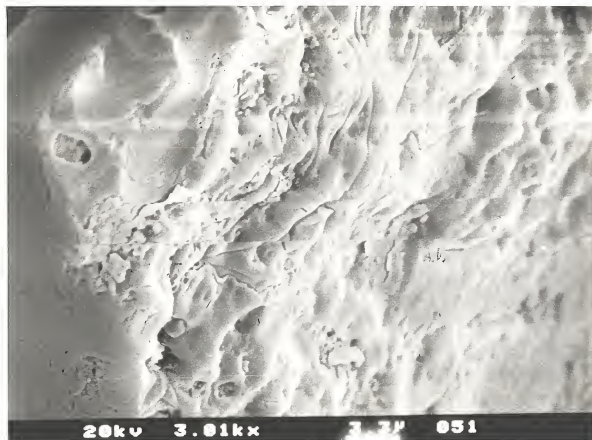


Figure 3-2. Scanning electron micrograph of acid-leached CP6-86; 3010X magnification.

Comparison of Silica and CPG Bonding Densities via Reflux
and Ultrasonic Syntheses

The controlled pore glasses of both pore sizes (denoted CPG-86 and CPG-167) were derivatized under the same types of reaction conditions as for the Davisil silica. Two aspects in particular were to be examined by these experiments; the first was to determine the reactivity of CPG compared to that of amorphous silica and the second was to determine the effect of pore size on CPG reactivity. From pore shape indices, it has been found that the pore shape of siliceous pores is not usually cylindrical; therefore the cylindrical pore model is not a good approximation for silica (Nikolov, 1986). Since CPG has a very uniform pore diameter compared to that of silica and the CPG pores are much larger than the molecular dimensions of the reactive silane (the average molecular cross-sectional area for the octadecylchlorosilane is $50.95 \text{ (Angstroms)}^2$ per molecule and its length is 24.72 Angstroms (Cheng and McCown, 1985)) it was expected that the CPG's would exhibit higher reactivity and therefore result in alkyl bonding densities higher than those achieved with silica. In addition, it was expected that CPG-167 would be more reactive than CPG-86 due to its larger pore size. Other workers have found that octadecyl bonding density increases with increasing pore size for silica substrates (Engelhardt et al., 1982; Sander and Wise, 1984b; Sands et al., 1986; Staroverov et al., 1986).

In the first set of reactions, both CPG-86 and CPG-167 were rotated at ambient temperature (26.0 °C) for 24 hours

using dimethyloctadecylchlorosilane as the reactive silane, 2,6-lutidine as the acid-acceptor catalyst and methylene chloride as the reaction solvent. All bonding densities are calculated from duplicate elemental analyses as described in Chapter II and the mean value \pm the range is reported in all cases. The bonding densities for the CPG-86 and the CPG-167 were $2.56 \pm 0.03 \mu\text{mol}/\text{m}^2$ and $2.07 \pm 0.02 \mu\text{mol}/\text{m}^2$ respectively. In the second set of experiments, the same reagents and reaction time as above were used to react both CPG's, but the reactions were performed under reflux conditions at a temperature of 50.0°C . The CPG-86 bonding density was $2.63 \pm 0.00 \mu\text{mol}/\text{m}^2$ and that for the CPG-167 was $2.28 \pm 0.00 \mu\text{mol}/\text{m}^2$. As expected from previous work with silica (Chapter II), reflux temperatures resulted in a higher bonding density than ambient temperatures.

The next three sets of experiments were run for 24 hours with rotation under ultrasound conditions as described in Chapter II. The first set of experiments used the same reagents as described above at a temperature of 28.0°C . The CPG-86 had a resultant bonding density of $2.55 \pm 0.01 \mu\text{mol}/\text{m}^2$; that for the CPG-167 was $2.04 \pm 0.03 \mu\text{mol}/\text{m}^2$. As in the case for the silica, bonding densities achieved under ultrasonic conditions with 2,6-lutidine as the acid-acceptor catalyst were comparable to those achieved at ambient temperatures.

The second set of ultrasound experiments was run under the same conditions as the first set and at a temperature of

28.5 °C, except that 4-dimethylaminopyridine (4-DMAP) was used as the acid-acceptor catalyst instead of 2,6-lutidine. The bonding densities for CPG-86 and CPG-167 were 3.30 ± 0.02 and $3.04 \pm 0.08 \mu\text{mol}/\text{m}^2$ respectively. The 4-DMAP had again proven to be a superior acid-acceptor catalyst to the 2,6-lutidine as had been the case for silica. The third set of ultrasound experiments was performed at 28.0 °C using 4-DMAP as the acid-acceptor catalyst, methylene chloride as the reaction solvent and trimethylchlorosilane (TMCS) as the reactive silane. TMCS was used in order to approximate the maximum bonding density achievable under minimum steric hindrance conditions, as explained in Chapter II. The TMCS bonding density was $4.19 \pm 0.02 \mu\text{mol}/\text{m}^2$ for CPG-86 and $5.15 \pm 0.10 \mu\text{mol}/\text{m}^2$ for CPG-167. As expected, use of a less bulky silane reagent resulted in a higher alkyl bonding density, since steric hindrances are minimized.

A comparison of the bonding densities of the 147 Angstrom pore size silica, 86 Angstrom CPG and 167 Angstrom CPG achieved under all sets of conditions is tabulated in Table 3-1. As seen from these results, neither of our expectations was realized. In all of the octadecyl silane reactions, reactivity of the amorphous silica was greater than that of either CPG. Even more puzzling, the smaller pore CPG (CPG-86) exhibited greater reactivity than the wider pore CPG (CPG-167). In the case where TMCS was the reactive silane, these trends were reversed. This seems to indicate that the reactivity trends for the silane bonding

Table 3-1. Comparison of octadecyl bonding densities for 147 Angstrom (pore size) silica, 86 Angstrom controlled pore glass (CPG-86) and 167 Angstrom controlled pore glass (CPG-167).

Reaction Conditions ¹	Temperature (°C)	C ₁₈ Bonding Density (μmol/m ²)		
		silica	CPG-86	CPG-167
Refluxed/ 2,6-lutidine	50.0	2.82	2.63	2.28
Ultrasound/ 2,6-lutidine	28.0	2.71	2.55	2.04
Ultrasound/ 4-DMAP	28.5	3.35	3.30	3.04
Ambient/ 2,6-lutidine	26.0	2.69	2.56	2.07
C ₁ silane ² / Ultrasound/ 4-DMAP	28.0	3.51	4.19	5.15

¹ Reaction method/acid-acceptor catalyst.

² C₁ silane used instead of C₁₈ silane in order to estimate bonding density achievable using a less bulky silane reagent.

reactions could be due to steric problems, since TMCS is much smaller than dimethyloctadecylchlorosilane, but the lower reactivity of the CPG-167 compared to that of CPG-86 for the octadecyl reaction contradicts this theory. The chemical composition of the CPG's may be implicated in this problem, since CPG contains 3% B_2O_3 and silica contains no more than trace amounts. Other workers have found that heating CPG to a temperature of 700 °C for 5 to 100 hours results in migration of boron atoms to the glass surface and consequent enrichment of boron on the surface of the porous glass. They further found that this surface boron enrichment resulted in higher octadecyl bonding densities than achieved on untreated CPG (Dawidowicz et al., 1983; Dawidowicz et al., 1986; Dawidowicz and Rayss, 1985; Rayss et al., 1983; Rayss and Dawidowicz, 1986; Suprynowicz et al., 1985). At present, we are unable to explain the anomalous octadecyl bonding behavior of the controlled pore glasses.

CHAPTER IV
CORRELATIONS BETWEEN CHROMATOGRAPHIC RETENTION AND OCTADECYL
BONDING DENSITY

Chromatographic Determination of Thermodynamic Partition
Coefficients

Retention in any chromatographic process occurs when the solute of interest is transferred from the mobile phase to the stationary phase. The process of transfer of the solute between the mobile phase and the stationary phase is characterized by the thermodynamic distribution or partition coefficient, K , which is the ratio of the concentration of the solute in the stationary and mobile phase.

Chromatographic retention is a function of the distribution coefficient and the volumes of the respective phases, and is most often described by the capacity factor, k' , the ratio of the number of moles of solute in the stationary phase and in the mobile phase. The capacity factor also expresses the ratio between the amount of time the solute spends in the stationary phase and in the mobile phase. It is the commonly used measure to describe retention because it accounts for differences in column dimensions and mobile phase flow rates; it is also easy to measure as $k' = (V_r - V_m)/V_m$ where V_r is the solute retention volume and V_m is the mobile phase void volume. Measurement of the capacity factor provides valuable thermodynamic information

about solute retention in a particular chromatographic system since retention is related to the thermodynamic distribution coefficient through the volume phase ratio V_s/V_m (stationary/mobile) in that $k' = K(V_s/V_m)$. Therefore for rigorous theoretical treatment of chromatographic retention, the phase ratio must be accurately known in order to determine the partition coefficient.

Measurement of the Mobile Phase Volume in RPLC

The determination of the mobile phase volume, V_m , in liquid chromatographic systems is a problem that has generated great interest as well as considerable controversy. Its value is an essential component for the calculation of the capacity factor k' as well as for the thermodynamic distribution coefficient K . Many workers have addressed this dilemma, yet there is little consensus on a generally applicable convention for measuring V_m (Berendsen et al. 1980b; Engelhardt et al., 1984; Gutnikov and Hung, 1984; Knox and Kaliszan, 1985; Le Ha et al., 1982; McCormick and Karger, 1980; Melander et al., 1983a and 1983b; Slaats et al., 1981; Wainwright et al., 1985; Wells and Clark, 1981). The problem is especially complex for RPLC, since preferential sorption of mobile phase components by the stationary phase results in the formation of a solvation layer on this surface. The thickness and composition of the solvation layer varies with the bulk composition of the mobile phase and the local concentration of organic modifier in the solvation layer may be greater than that in the bulk

mobile phase due to the hydrophobicity of the stationary phase. The composition of the solvation layer also varies with its distance from the anchored ends of the RP chains; therefore its presence results in an ill-defined boundary between the stationary and mobile phases (Berendsen et al. 1980b; Gutnikov and Hung, 1984; Le Ha et al., 1982, Knox and Kaliszan, 1985).

There are three general categories of procedures used to determine V_m : the use of unretained compounds, the linearization of the net retention time for a homologous series and static methods (Berendsen et al., 1980b). The choice of an "unretained" compound for V_m measurements in RPLC systems is a difficult one. In any case, neither its heat of sorption nor its size should differ from those of the mobile phase components. For this reason, mobile phase constituents and especially their deuterated analogs are often used (Engelhardt et al., 1984). However, this choice is not without its problems. Since these compounds are transparent in the UV region, sensitive detection of them requires a refractive index detector. The use of deuterated organic modifier as an unretained compound is only valid when there is a large amount of organic modifier present in the mobile phase since in organic-lean mobile phases the marker will be slightly retained, especially in the case of methanol/water mobile phase systems (Engelhardt et al., 1984). The same is true for D_2O in organic-rich mobile phase systems; this phenomenon is attributed to D_2O

adsorption onto the residual silanol groups on the stationary phase surface (McCormick and Karger, 1980). Melander et al. (1983a, p. 213) suggest that " . . . the most weakly bound solvent component is not present in the solvation layer." and that this component should be used for the determination of V_m . They concur with McCormick and Karger (1980) that D_2O is a useful probe for mobile phase volume determination unless the mobile phase is water-lean. As secondary probes of V_m , fructose and urea have been suggested for all compositions of methanol/water mobile phases and for acetonitrile volume fractions from 0 to 0.75 for acetonitrile/water mobile phase (Melander et al., 1983a). Gutnikov and Hung (1984) have also proposed the use of oxalohydroxamic acid as a UV-detectable V_m probe.

The use of UV-active inorganic salts such as nitrates has also been recommended for determination of RPLC dead volumes; however in unbuffered mobile phases the dead volumes obtained increase with increasing amount of salt injected. Nitrate is also prevented from penetrating the stationary phase pores by the Donnan potential which comes about from the negatively charged silicate ions present on the stationary phase surface (Berendsen et al., 1980b; Engelhardt et al., 1984; Wells and Clark, 1981). Knox and Kaliszan (1985) suggest using a volume fraction-weighted average of the retention volumes of isotopically labelled forms of the mobile phase components. However, besides the aforementioned problems associated with the detection of

these species, this method adopts the convention that V_m is the total volume of all mobile phase components within the column bed; the solvation layer adsorbed on the stationary phase is thereby included in the mobile phase volume.

The linearization of a homologous series of compounds in order to find the column dead volume has been widely used in gas chromatography; therefore it is not surprising that this method has also been applied for LC systems. This method assumes that there is a linear relationship between the logarithm of the net retention time t_r and the carbon number of a homologous series (Berendsen et al., 1980b; Laub and Madden, 1985; Wainwright et al., 1985). The mobile phase volume can be obtained by comparing the retention times for two consecutive homologs, termed n and $n+1$. For a homologous series the ratio of capacity factors for consecutive homologs is assumed to be constant and

$$t_{r,n+1} = A(t_{r,n}) - (A - 1)t_0.$$

By plotting $t_{r,n+1}$ versus $t_{r,n}$, the slope A can be obtained and t_0 can be determined from the intercept and multiplied by the mobile phase volume flow rate to obtain V_m . The results obtained can be precise to within 1% if alkyl-benzenes are the homologous series used (Berendsen et al., 1980b); however the use of homologous aromatic alcohols gives inconsistent data due to their interactions with silanol groups on the stationary phase (Laub and Madden, 1985).

The linearization method is not without criticism. It assumes that the relationship between the logarithm of the

retention times for a homologous series is a linear function of the number of carbons in the series and therefore that the change in free energy of partitioning per methylene group is constant. This implies that as the number of methylene groups increase, the rest of the molecule has a constant effect on stationary phase interactions. Yet in some cases the relationship between the logarithm of retention and carbon number is not linear, implying that this assumption is invalid. The linearization method is also very time consuming, since the retention time measurements must be determined very precisely in order to obtain precision in the V_m value (Knox and Kaliszan, 1985).

Determination of V_m by the static method is a gravimetric procedure. A thermostatted packed LC column is filled successively with two pure liquids with greatly different densities and weighed. From the differences in the column masses, w , and the density of each liquid, d , the total mobile phase volume can be calculated, since

$$V_m = \frac{w_a}{d_a} - \frac{w_b}{d_b}$$

(Berendsen et al., 1980b; Knox and Kaliszan, 1985; McCormick and Karger, 1980). The mobile phase volume as determined by this method is the maximum volume within the column that is accessible to a molecule comparable in size to those used in the procedure (McCormick and Karger, 1980). However, this method ignores the possibility of a solvation layer on the stationary phase and therefore can overestimate the value of a dynamic V_m by as much as 15% for a pure methanol mobile

phase (Berendsen et al., 1980b). The volume of the mobile phase as determined by this method is useful for a reference point, both in terms of whether or not a compound experiences retention in a chromatographic system and in understanding the changes in the solvation layer which occur when the bulk mobile phase composition is changed (McCormick and Karger, 1980). Knox and Kaliszan (1985) even argue that in the theoretical treatment of thermodynamic aspects of chromatography that the maximum V_m value is the pertinent one. They argue that since the thickness of the boundary between the bulk stationary phase and bulk mobile phase is on the order of one nanometer that its position cannot be sufficiently well defined to give an accurate measure of the two volumes and that calculational methods for the volume of the solvation layer are very arbitrary. Because the gravimetric procedure gives a precise and reproducible value for V_m that is unambiguous and convenient to measure, this convention was chosen to determine the mobile phase volume for the chromatographic thermodynamic distribution coefficients calculated in this work.

Measurement of the Stationary Phase Volume in RPLC

Although much work has been done on measuring the volume of the mobile phase, measurement of V_s , the volume of the stationary phase has not been as thoroughly investigated (Berendsen et al., 1980b; Jandera et al., 1982; McCormick and Karger, 1980; Melander et al., 1980; Sander and Field, 1980; Slaats et al., 1981). In determining a method for the

measurement of V_s , a convention for defining V_s must be chosen, since the choice of the phase ratio must be compatible with the definition of K that is in agreement with the molecular mechanism of retention. Jandera et al. (1982) have defined the stationary phase volume as that fraction of the column volume that is not occupied by the mobile phase. While this choice is certainly convenient and can be readily determined, it is at best a crude measure, as similar (or even identical) values of V_s would be obtained for stationary phases made from the same bulk silica but with different bonding densities of alkyl chains, or possibly even of different chain lengths. Any determination of stationary phase volume based solely on mobile phase volume measurements is doomed to failure, as such a measurement cannot be sufficiently sensitive to ascertain bonding density or small chain length differences.

Melander and Horvath (1980) have suggested defining the phase ratio as the ratio of the surface area of the adsorbent (m^2) divided by the column dead volume (cm^3). While this approach is an improvement in definition, it again fails to account for certain variations in the structure of the bonded phase and it implies that adsorption is the sole mechanism in RPLC retention. The major drawback to this proposed phase ratio convention, however, lies in the accurate measurement of the two parameters involved. As previously mentioned, chromatographers have been unable to embrace any one of the commonly used methods for determining

column dead volumes as being accurate and consistent enough for precise work (Engelhardt et al., 1984; Melander et al., 1982; Smith et al., 1986). Melander and Horvath (1980) state that a small relative error in the determination of the column dead volume results in a commensurate relative error in calculating both the capacity factor and the Gibbs free energy of the solute transfer.

Other problems exist as well. The surface area of the adsorbent is usually found by use of the BET analysis method. It should be noted that the surface area of the adsorbent must be determined after derivatization with the alkyl ligand, as the surface area of the derivatized silica will be significantly different from that of the underivatized support. Although use of the BET method for surface area determination is widespread, this method is inappropriate in assessing that surface area of derivatized silica packings which is chromatographically significant. The BET method measures the area of surface that is accessible to a small molecular probe such as nitrogen. Yet in an irregular surface such as porous silica, there may exist many pores which are large enough to allow nitrogen in, but which are too small to allow the passage of any larger molecules of chromatographic interest. Chromatographic support surface area data based on BET analysis is usually overestimated, and the amount of overestimation is by no means a constant, depending on the base silica structure and the derivatization method.

Melander and Horvath (1980, p. 270) state that ". . . any estimation of "stationary phase volume" on the basis of BET surface area of the support is likely to be inaccurate." Due to the errors in determining both adsorbent surface area and column dead volume, there will consequently be a large error propagated in the subsequent calculation of the phase ratio if Melander and Horvath's convention is used.

Sander and Field (1980) have estimated the phase ratio by constructing physical models of the bonded phase using manufacturer's data regarding silanol surface coverage and percent carbon loading. This approach is quite reasonable from a theoretical standpoint, as it accounts for variation in bonding density and alkyl chain length. However because it is based on models it can only be an estimate of V_s ; the construction of such physical models is also time consuming.

In determining the stationary phase volume, the pertinent volume should be the volume of the alkyl chains bonded to the silica surface. Dill (1987a) has performed statistical mechanical calculations based on a lattice interphase model of RPLC stationary phases which describe chromatographic retention in reversed phase systems. These calculations have shown that in a well endcapped column, chain interactions with solutes are the most important stationary phase contribution to solute retention. Therefore the calculation of V_s should give only the actual volume of the alkyl chains bonded to the support surface. The assumption here is that all of the bonded stationary

phase volume is accessible to the solute.

A simple method for calculating the stationary phase volume has been devised in our laboratory. The only measurements necessary are the carbon load of the packing and the actual weight of packing contained in the chromatographic column. Wise and May (1983) proposed that the surface density of a bonded alkyl ligand (in micromoles of alkyl ligand per square meter of packing surface) can be calculated by

$$C_s = \frac{\%C (10^6)}{1200 n_c S_{BET}} \quad (4-1)$$

where C_s is the bonding density ($\mu\text{mol}/\text{m}^2$), $\%C$ is the carbon loading of the packing as determined from elemental analysis or by a gravimetric procedure (Cheng, 1985), n_c is the number of carbons in the alkyl ligand, and S_{BET} is the surface area of the derivatized packing as determined by BET analysis. But the volume of the stationary phase, V_s , can be expressed as

$$V_s = (C_s)(S_{BET})(v)(W_p)(10^{-6}) \quad (4-2)$$

where v is the molar volume of the bonded alkyl group in cm^3/mole and W_p is the weight of the bonded packing contained in the chromatographic column. Molar volume, v , is

$$v = M/d \quad (4-3)$$

where M is the weight of the bonded phase alkyl group and d is the density of the bonded alkyl group. Cheng (1985) has experimentally determined the pertinent densities of commonly used bonded silanes and reported values of 0.8607

g/cm³, 0.8625 g/cm³, and 0.8638 g/cm³ respectively for the octadecyldimethylsilyl, octyldimethylsilyl, and trimethylsilyl bonded groups. Substitution of equations 4-1 and 4-3 into equation 4-2 results in the volume of the stationary phase, V_s (in cm³), as expressed by the following formula

$$V_s = \frac{(\%C)(M)(W_p)}{(100)(12.011)(n_c)(d)} \quad (4-4).$$

This method provides a much more accurate calculation of V_s than has been previously possible. A principal advantage of this method is that the surface area of the packing is not used in determining V_s , which eliminates the errors associated with this measurement. The stationary phase volume that is calculated in this method is the volume that is important in the chromatographic process, i.e. the actual volume of the bonded alkyl chains themselves. The precision is limited only by the carbon loading determination (± 0.20 %C for our departmental elemental analysis) and by the measurement of the mass of packing in the column (± 0.1 mg on any analytical balance). Calculation of the volume of the stationary phase by this method provides the means for a more accurate and uniform determination of the phase ratio.

Experimental Procedure

Preparation of Bonded Phases of Varied Bonding Densities

All of the reagents used in the preparation of the silica and CPG bonded phases are described in Chapters II and III. Silica bonded phases with octadecyl bonding

densities greater than or equal to $2.75 \mu\text{mol}/\text{m}^2$ were prepared under traditional reflux conditions as well as under ambient, subambient and superambient ultrasonic conditions, using 2,6-lutidine or 4-DMAP as the acid-acceptor catalyst as described in Chapter II. Controlled pore glass bonded phases with octadecyl bonding densities of 3.30 and $2.63 \mu\text{mol}/\text{m}^2$ were prepared from the 86 Angstrom CPG (CPG-86) under ambient ultrasonic conditions using 4-DMAP as the acid-acceptor catalyst or under reflux conditions using 2,6-lutidine as the acid-acceptor catalyst respectively as described in Chapter III. Table 4-1 lists the experimental conditions, acid-acceptor catalyst and resultant octadecyl bonding density for each of these stationary phases.

In order to prepare bonded phases with octadecyl bonding densities less than $2.6 \mu\text{mol}/\text{m}^2$, the experimental conditions of the bonding reaction must be altered so that a less than maximal bonding density is achieved. One strategy that can be used to accomplish this is to use a less than stoichiometric amount of the reactive silane. Another approach is to partially cover some of the reactive silanols with trimethylsilane before exhaustive derivatization with the octadecyl silane reagent (Marshall et al., 1984 and 1986). By varying the amount of trimethylsilane precoverage and then reacting the precovered silicas with an excess of the octadecylsilane, lower coverage octadecyl bonded phases of varying bonding densities can be synthesized.

Table 4-1. High octadecyl bonding density reversed phase packings.

Packing Identifier	Base Packing	Reaction Conditions	Temperature (°C)	Reaction Time (h)	Acid-Acceptor Catalyst	C ₁₈ Bonding Density (μmol/m ²)
C18-2	silica	reflux	50.0	24	2,6-lutidine	2.75
LT2	silica	ultrasound	8.5	101	2,6-lutidine	2.84
DMAP1	silica	ultrasound	28.0	24	4-DMAP	3.06
DMAP1/3 ¹	silica	ultrasound	28.0/4.0	24/97	4-DMAP	3.15
DMAP3	silica	ultrasound	4.0	97	4-DMAP	3.24
US/ref1	silica	ultrasound	50.0	24	4-DMAP	3.34
ref/DMAP	silica	reflux	50.0	24	4-DMAP	3.43
rederDMAP1 ²	silica	ultrasound	28.0	24	4-DMAP	3.56
DMAP5	silica	ultrasound	3.0	144	4-DMAP	3.60
CPG2	CPG-86	reflux	50.0	24	2,6-lutidine	2.68
CPG4	CPG-86	ultrasound	28.5	24	4-DMAP	3.30

¹ Packing DMAP1/3 is a 50/50 (weight/weight) mixture of packings DMAP1 and DMAP3.

² Packing rederDMAP1 was obtained by reacting packing DMAP1 with the octadecyl silane under identical conditions as for DMAP1.

Based on Kinkel and Unger's (1984) estimation of a maximum of five micromoles of reactive hydroxyl sites per square meter of silica surface, amounts of trimethylchlorosilane (TMCS) corresponding to approximately 5%, 10%, 15%, 30% and 40% coverage of these hydroxyl sites were reacted as described in Chapters II and III under ambient conditions (at a temperature of 26.5 °C) for 24 hours to partially precover the silica and controlled pore glass supports. The reaction solvent was dry methylene chloride and 2,6-lutidine was used as the acid-acceptor catalyst. It should be noted that it is unlikely that exactly 5%, 10%, 15%, 30% or 40% of the surface hydroxyl groups on the supports were reacted; the amounts of TMCS used merely represent some fraction of the amount necessary for total coverage of the surface (Marshall et al., 1984). After TMCS precoverage, the supports were washed and dried as described in Chapters II and III. The precovered supports were then reacted with a twofold excess of octadecyldimethylchlorosilane and a fourfold excess of 2,6-lutidine with methylene chloride at a temperature of 26.5 °C for 24 hours and washed and dried as described in Chapters II and III. The resultant C₁ and C₁₈ bonding densities for these precovered bonded phases are summarized in Tables 4-2 and 4-3.

HPLC Column Packing Procedures

HPLC columns were assembled from 15 cm lengths of 1/4" outer diameter and 4.6 mm inner diameter seamless precision bore polished HPLC tubing (Alltech Associates, Inc.,

Table 4-2. Bonding densities for precovered silica reversed phase packings.

Packing Identifier	C ₁ Bonding Density ($\mu\text{mol}/\text{m}^2$)	C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)
5%	0.63	2.07
10%	0.98	2.09
15%	1.50	1.98
30%	1.38	1.74
40%	1.90	1.60

Table 4-3. Bonding densities for precovered 85 Angstrom controlled pore glass reversed phase packings.

Packing Identifier	C ₁ Bonding Density ($\mu\text{mol}/\text{m}^2$)	C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)
5% CPG	0.55	3.21
10% CPG	1.08	2.83
15% CPG	2.25	1.70
30% CPG	1.27	2.72
40% CPG	1.34	2.59

Deerfield, IL) and from Swagelok 1/4" to 1/16" zero dead volume reducing union chromatographic end fittings (Crawford Fitting Company, Solon, OH) which had been fitted with 2 μ m passivated 316 stainless steel frits (Alltech Associates, Inc., Deerfield, IL). The column tubing and end fittings were made of 316 stainless steel and were passivated prior to use by ultrasonication for 30 minutes in 3 M nitric acid, followed by an aqueous and a methanol rinse.

Silica columns were packed using a Shandon high pressure HPLC column packer with a 33 ml slurry reservoir (Shandon Southern Instruments, Inc., Sewickley, PA). Approximately 1.5 grams of derivatized silica were slurried in 30 ml of chloroform and sonicated for 10 minutes. The column was then packed at a packing pressure of 6000 psi in the downward position using a sequence of 150 ml each of 50/50 (v/v) chloroform/methanol, methanol and 50/50 methanol/water. The column was then removed from the packer fittings, the packing leveled with a spatula and the remaining column end fitting installed. Controlled pore glass columns were packed in an identical manner except for the type of packer used. Since CPG is mechanically fragile and brittle (Fluka), it cannot be packed using a high pressure packer. Therefore a Beckman Model 100A HPLC pump(Beckman Instruments Inc., San Ramon, CA) running at a flow rate of 9.9 ml/min was used in conjunction with continuous mechanical vibration to pack the CPG columns. Packing in such a manner generated a packing pressure of

200-300 psi, preserving the integrity of the CPG packings and resulting in a stable packing bed. All columns were equilibrated with the desired chromatographic mobile phase by passing approximately 125 ml of the mobile phase through the column immediately prior to use.

Chromatographic Measurements

The liquid chromatographic system used for chromatographic measurements consisted of a Valco C6W injector (Valco Instrument Company, Inc., Houston, TX) with a 10 microliter sample loop, a Beckman Model 100A isocratic HPLC pump, a Beckman Model 153 fixed wavelength 254 nm UV detector and a Fisher Recordall chart recorder (Fisher Scientific, Fairlawn, NJ). Samples were loaded in the injection loop via a Hamilton 705 SNR syringe (Reno, NV). Temperature control of the chromatographic column was accomplished by passing thermostatted water through a water jacket fitted around the column. Superambient temperatures were controlled using a Lauda Model MT heater/circulator (Brinkmann Instruments Company, Westbury, NY). Subambient temperatures were brought about by a Neslab Endocal 800 water bath (Neslab Instruments, Portsmouth, NH). Mobile phases were made of HPLC grade methanol or acetonitrile (Fisher Scientific, Fairlawn, NJ) mixed with water which had been prepared as described in Chapter II; mobile phase compositions are designated by volume ratios of organic modifier to water. Mobile phases were premixed by adding the appropriate volume of organic modifier to the

appropriate volume of water; these mobile phases were then mixed well and placed in an ultrasonic bath for 15-30 minutes in order to degas them. The flow rate of the mobile phase in all cases was 1.5 ml/min. Naphthalene (Eastman Organic Chemicals, Rochester, NY) was chosen as a small nonpolar test solute; standards were made up in HPLC grade methanol for use in the retention studies. Solute retention and column holdup volumes were measured from the chart recorder tracings. The solvent disturbance peak was used to determine column mobile phase volumes for the calculation of capacity factors; since this disturbance comes about from the methanol in which the test solute is dissolved, its choice for V_m falls under the category of using an unretained compound to determine V_m . This convention was chosen in order to account for variances in the individual column dead volumes resulting from differences in the stationary phase packing density within the chromatographic columns.

Measurement of V_m and V_s

The gravimetric procedure previously described was used to calculate V_m . While an ambient temperature of 25.0 °C was maintained, 150 ml of methylene chloride was passed through a 15 cm LC column packed with either silica or CPG. The column was then capped and weighed on an analytical balance. The procedure was duplicated using methanol as the mobile phase and by dividing the difference in column masses by the difference in the solvent densities at 25.0 °C (1.318

and 0.7866 g/cm^3 for methanol and methylene chloride respectively) the silica column dead volume was determined to be 1.805 ml while V_m for the CPG column was 1.752 ml. It was assumed that the mobile phase volumes measured by this procedure will be constant (within experimental error) for any of the silica or CPG reversed phase columns used in this work, since in each case the packings were based on the same starting material.

The stationary phase volume for each LC column was calculated using Equation 4-4. For the precovered bonded phases the total volume of both the trimethyl- and octadecylsilyl alkyl groups was used for V_s . Percent carbon for each column packing was obtained from in-house elemental analysis and the densities used for the trimethylsilyl and octadecylsilyl groups were 0.8638 and 0.8607 g/cm^3 , respectively as reported by Cheng (1985). The weight of the packing contained in the chromatographic column was determined by weighing an empty chromatographic column, packing it as described previously in this chapter and drying it at 100°C to constant weight in a gas chromatograph with a constant helium flow through the column. From the mass differences in the two weighings the weight of the column packing was determined to be 1.1705 g for the silica columns and 1.2898 for the CPG columns. The capacity factors for the naphthalene solute were determined by triplicate injections onto each of the chromatographic columns of different alkyl bonding density using mobile

phases consisting of 55/45 methanol/water and 85/15 acetonitrile/water. Thermodynamic partition coefficients for the transfer of the naphthalene solute from the stationary phase to the mobile phase were calculated by dividing the capacity factor by the volume phase ratio.

Results

Silica-based Stationary Phases

Thermodynamic partition coefficients for the naphthalene solute as a function of silica stationary phase octadecyl bonding density are listed in Tables 4-4, 4-5 and 4-6 respectively for the 55/45 methanol/water mobile phase system at 20.0 °C and 35.0 °C and for the 85/15 acetonitrile/water mobile phase system at 35.0 °C. Graphical representations of these data are shown in Figures 4-1, 4-2 and 4-3.

In all cases the partition coefficient increased as a linear function of bonding density until a bonding density of $3.1 \mu\text{mol}/\text{m}^2$ was reached. The best fit line for this linear region of each plot was calculated using least squares linear regression. For 55/45 methanol/water at 20.0 °C the slope and y-intercept for the best fit line were 30.1 and 12.3 respectively with a coefficient of correlation of 0.989. At 35.0 °C for 55/45 methanol/water the slope was 21.0, the y-intercept 12.7 and the coefficient of correlation 0.973. For the 85/15 acetonitrile/water mobile phase at 35.0 °C the slope was 1.20, the y-intercept 1.54 and the coefficient of correlation 0.982. In all cases the

Table 4-4. Naphthalene thermodynamic partition coefficients at 20.0 °C as a function of silica octadecyl bonding density for 55/45 methanol/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	Naphthalene Thermodynamic Partition Coefficient at 20.0 °C
1.60	57.6
1.74	66.7
1.98	69.6
2.07	78.6
2.09	75.8
2.75	94.3
2.84	96.2
3.06	104
3.15	97.2
3.24	93.4
3.34	89.6
3.43	87.2
3.56	86.1
3.60	85.9

Table 4-5. Naphthalene thermodynamic partition coefficients at 35.0 °C as a function of silica octadecyl bonding density for 55/45 methanol/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	Naphthalene Thermodynamic Partition Coefficient at 35.0 °C
1.60	43.4
1.74	50.7
1.98	51.3
2.07	60.5
2.16	59.5
2.75	69.8
2.84	71.3
3.06	76.1
3.15	70.9
3.24	69.2
3.34	66.8
3.43	65.0
3.56	63.9
3.60	63.7

Table 4-6. Naphthalene thermodynamic partition coefficients at 35.0 °C as a function of silica octadecyl bonding density for 85/15 acetonitrile/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	Naphthalene Thermodynamic Partition Coefficient at 35.0 °C
1.60	3.28
1.74	3.69
1.98	3.79
2.07	4.20
2.09	4.16
2.75	4.90
2.84	4.95
3.06	5.09
3.15	4.88
3.24	4.73
3.34	4.66
3.56	4.48
3.60	4.41

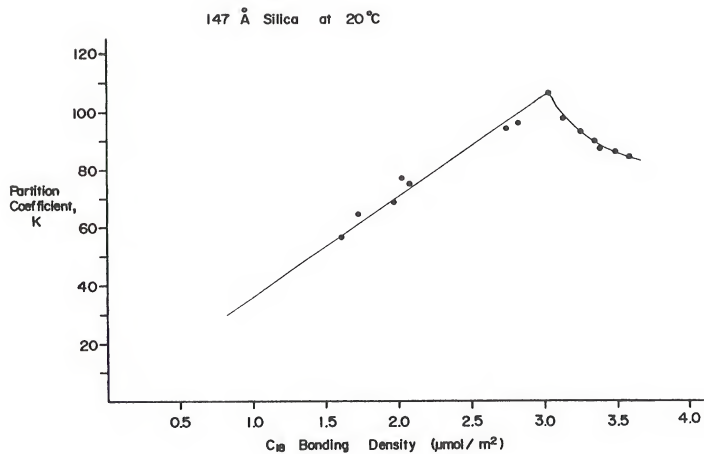


Figure 4-1. Naphthalene thermodynamic partition coefficient at 20.0 °C as a function of silica octadecyl bonding density for 55/45 methanol/water mobile phase.

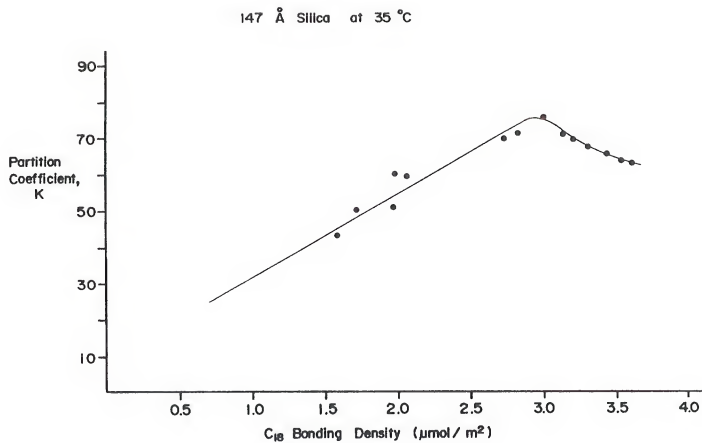


Figure 4-2. Naphthalene thermodynamic partition coefficient at 35.0 °C as a function of silica octadecyl bonding density for 55/45 methanol/water mobile phase.

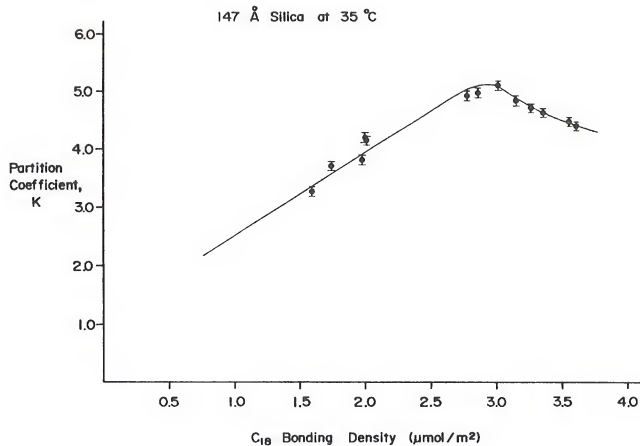


Figure 4-3. Naphthalene thermodynamic partition coefficient at 35.0 °C as a function of silica octadecyl bonding density for 85/15 acetonitrile/water mobile phase.

coefficient of correlation showed a definite linear relationship between partition coefficient and bonding density, especially in light of the uncertainties in the measurements of bonding density, V_s , V_m and capacity factor. The amount of error propagated in the bonding density could be calculated from the variances in percent carbon and support surface area; similarly, that for the stationary phase volume (as calculated by Equation 4-4) could be calculable from the variances in percent carbon, weight of the packing contained in the column and density of the alkyl group bonded to the silica surface. However, these calculable sources of error are small when compared to the incalculable sources of error in the stationary and mobile phase volumes. The primary source of error in V_m determination is the choice of convention for its measurement, as discussed earlier in this chapter. In fact the choice of V_m convention is the most significant source of error in the calculation of capacity factors as well, since $k' = (V_r - V_m)/V_m$, where V_r is the retention volume of the solute of interest. The most significant error in V_s is certainly the determination of the volume of the solvation layer associated with the stationary phase surface. As explained earlier in this chapter, we have developed an equation (4-4) to calculate the stationary phase volume which gives an accurate volume for the alkyl chains bonded to the silica surface; however this convention does not take the solvation layer volume into account. This omission was

deliberate because the solvation layer volume is very difficult to measure experimentally, moreover it changes substantially as the mobile phase composition is changed (Berendsen et al., 1980b; McCormick and Karger, 1980). Our calculations of V_s may therefore underestimate the true stationary phase volume, which will encompass the solvation layer volume as well as the bonded chain volume. Moreover, our static V_m value is larger than those calculated by most dynamic means since the static value includes the solvation layer volume in the mobile phase volume. The combination of these two effects results in an overall value for the volume phase ratio (stationary/mobile) that is smaller than the "true" value; consequently the solute thermodynamic partition coefficient as determined chromatographically will probably be overestimated, but the exact amount of overestimation is incalculable at this time. Although this possible overestimation of the partition coefficient would cause the plots of partition coefficient versus bonding density in Figures 4-1, 4-2 and 4-3 to be shifted vertically, it should be noted that this would change neither the shape of the plots nor their maxima. Error bars based on the range of partition coefficient values resulting from the imprecision in the elemental analysis for the silica packings ($\pm 0.20\%$ carbon) are shown for Figure 4-3.

At bonding densities greater than about $3.1 \mu\text{mol}/\text{m}^2$ the partition coefficient begins to decrease as the bonding density is increased. This trend is evident for both mobile

phase systems and/or temperatures. The overall behavior under the conditions specified above was that the partition coefficient increased linearly as a function of bonding density until a maximum was reached at a certain "critical" bonding density in the vicinity of $3.1 \mu\text{mol}/\text{m}^2$. Once this critical bonding density is reached, the earlier trend is reversed and the partition coefficient decreases with increasing bonding density. Comparisons between these experimental trends and Dill's (1987a and 1987b) theoretical predictions are made later in Chapter VI. These comparisons result in the interpretation of the experimental results, giving a wealth of information about the fundamental mechanisms of small solute retention in reversed phase liquid chromatographic systems.

Controlled Pore Glass-based Stationary Phases

Naphthalene thermodynamic partition coefficients as a function of CPG stationary phase octadecyl bonding density for 55/45 methanol/water at 20.0 °C and 35.0 °C and for 85/15 acetonitrile/ water mobile phase systems at 35.0 °C are listed in Tables 4-7, 4-8 and 4-9 respectively. Graphical representations of these data are shown in Figures 4-4, 4-5 and 4-6.

In all three cases the partition coefficient reached a local maximum at about $2.7 \mu\text{mol}/\text{m}^2$. For the 55/45 methanol/water mobile phase, at bonding densities higher than this value the partition coefficient decreased to a local minimum at approximately $2.8 \mu\text{mol}/\text{m}^2$ for a temperature

Table 4-7. Naphthalene thermodynamic partition coefficients at 20.0 °C as a function of CPG-86 octadecyl bonding density for 55/45 methanol/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	Naphthalene Thermodynamic Partition Coefficient at 20.0 °C
1.70	89.3
2.68	103
2.72	95.2
2.83	93.6
3.21	98.1
3.30	102

Table 4-8. Naphthalene thermodynamic partition coefficients at 35.0 °C as a function of CPG-86 octadecyl bonding density for 55/45 methanol/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	Naphthalene Thermodynamic Partition Coefficient at 20.0 °C
1.70	69.3
2.68	75.1
2.72	70.0
2.83	67.1
3.21	67.0
3.30	69.7

Table 4-9. Naphthalene thermodynamic partition coefficients at 35.0 °C as a function of CPG-86 octadecyl bonding density for 85/15 acetonitrile/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	Naphthalene Thermodynamic Partition Coefficient at 35.0 °C
1.70	11.0
2.59	13.5
2.68	15.0
2.72	13.9
2.83	11.9
3.21	9.76
3.30	9.74

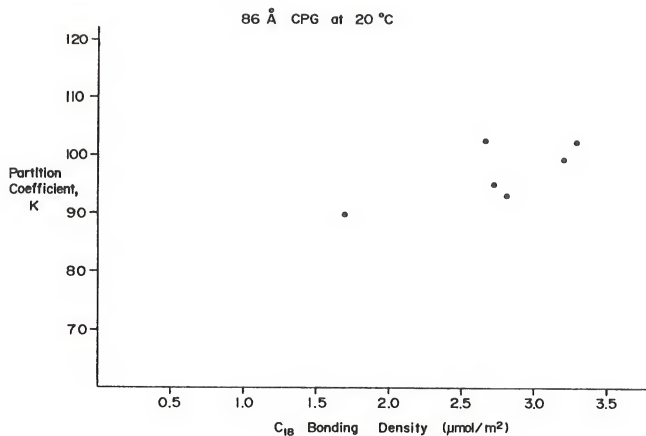


Figure 4-4. Naphthalene thermodynamic partition coefficient at 20.0 °C as a function of CPG-86 octadecyl bonding density for 55/45 methanol/water mobile phase.

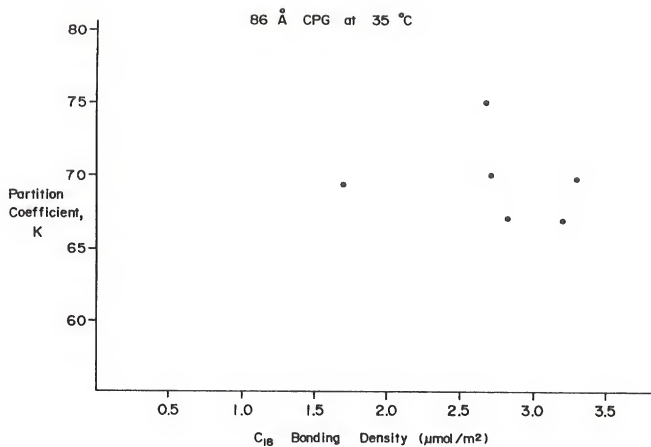


Figure 4-5. Naphthalene thermodynamic partition coefficient at 35.0 °C as a function of CPG-86 octadecyl bonding density for 55/45 methanol/water mobile phase.

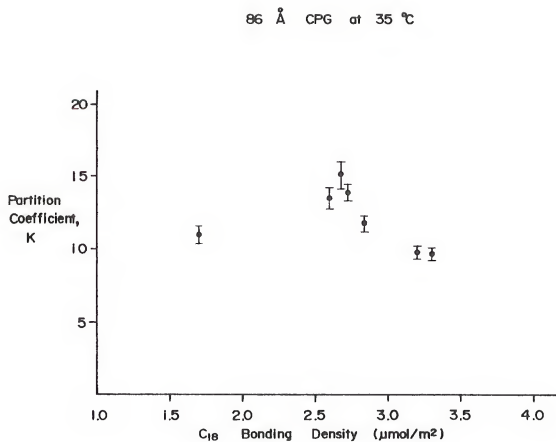


Figure 4-6. Naphthalene thermodynamic partition coefficient at 35.0 °C as a function of CPG-86 octadecyl bonding density for 85/15 acetonitrile/water mobile phase.

of 20.0 °C and in the area of 2.8 to 3.2 $\mu\text{mol}/\text{m}^2$ at 35.0 °C. At bonding densities higher than the local minimum the partition coefficient shows an increasing trend. This increasing trend contrasts with the behavior of the silica bonded phases, where the partition coefficient continuously decreases for bonding densities greater than about 3.1 $\mu\text{mol}/\text{m}^2$. In the 85/15 acetonitrile/water mobile phase system the partition coefficient exhibited a decreasing trend with increasing octadecyl bonding density once the local maximum at 2.7 $\mu\text{mol}/\text{m}^2$ is reached; this trend is similar to that exhibited by the silica bonded phases.

It is premature at this point in time to make any further comparisons between the behavior of the CPG bonded phases and that of the silica bonded phases as regards the relationship between bonding density and partition coefficient. The fourteen silica packings evaluated span a bonding density range from 1.60 to 3.60 $\mu\text{mol}/\text{m}^2$ while the seven CPG packings span a range of 1.70 to 3.30 $\mu\text{mol}/\text{m}^2$, with four of the seven packings in the narrow range of 2.6 to 2.8 $\mu\text{mol}/\text{m}^2$. Since there is such a small number of data points for the CPG packings, the scatter plots shown in Figures 4-4 through 4-6 are rather speculative and therefore can serve as only the most preliminary indication of the partitioning behavior of the CPG packings.

The sources of error in the calculations of capacity factor, V_s , V_m and partition coefficient have previously been discussed for the silica bonded phases; these errors

are also valid for the CPG bonded phases. In addition, the retention volumes of the naphthalene solute are difficult to measure precisely from their resultant chromatographic peaks. Since the CPG has a particle size range of 37 to 74 μm , the solute peaks are very broad and therefore the peak maxima are difficult to pinpoint precisely. The solvent disturbance peak was too indistinct and ambiguous for use in measuring V_m ; therefore V_m was determined by injection of D_2O . This should result in a fairly accurate V_m measurement for the 55/45 methanol/water mobile phase, but this method probably overestimates V_m for the 85/15 acetonitrile/water mobile phase since this is a water-lean system (McCormick and Karger, 1980; Melander, et al., 1980). Because of these considerations, measurements of solute capacity factors for the CPG bonded phases are even less accurate than those for the silica bonded phases.

The calculated bonding densities for the CPG packings are less precise than those for the silica packings due to imprecision in their elemental analysis for percent carbon. The analyst measuring the percent carbon in these packings (Courtney, 1987) had expressed his concern for the elemental analysis precision because the CPG packings were harder to weigh accurately due to handling difficulties as well as being susceptible to incomplete combustion during the analysis. These concerns were reflected in the precision of the percent carbon analysis for the CPG packings; for any given set of elemental analyses for a single CPG sample a

much wider range of percent carbon values was obtained than in an analogous situation with silica packings. In the worst case situation, the standard deviation in the percent carbon analysis was $\pm 0.61\%$ carbon. Error bars based on the range in the partition coefficient values which would result from this worst case standard deviation in the percent carbon value are shown for Figure 4-6. Comparison of Figure 4-6 to Figure 4-3 demonstrates that even the errors due to uncertainty in the elemental analysis are much more substantial for the CPG packings than for the silica packings. Additionally, due to the abovementioned problems there are no doubt greater errors in the values for octadecyl bonding density, capacity factor and V_m for CPG packings than for silica packings. Therefore it must be again stressed that the data depicting the behavior of the thermodynamic partition coefficient with respect to octadecyl bonding density for the CPG bonded phases is tentative at best and should be regarded as only a most preliminary prediction of retention characteristics for CPG chromatographic systems.

CHAPTER V
CORRELATIONS BETWEEN CHROMATOGRAPHIC SELECTIVITY AND ALKYL
BONDING DENSITY

Introduction

Chromatographic selectivity (α) is the difference in retention between two solute molecules. Chromatographic selectivity is an important thermodynamic measurement in studies of the solute distribution process since it is directly related to the difference in the Gibbs free energy of transfer from the mobile phase to the stationary phase for two solutes:

$$\ln \alpha = - \Delta(\Delta G)/RT ,$$

where ΔG is the Gibbs free energy, R is the gas constant, and T is the absolute temperature. Consequently, any two solutes possessing different free energies of transfer will be differentially retained (Lochmuller et al., 1985; Melander and Horvath, 1982). Selectivity between two solutes is measured as the ratio of their capacity factors; $\alpha_{a,b} = k_a' / k_b'$, and is defined such that $\alpha \geq 1.0$. Functional group selectivity is the change in retention for a given solute caused by the addition (or subtraction) of a particular functional group. Evaluation of functional group selectivity is accomplished by measuring the capacity factors for a homologous series of compounds which differ

from each other by the functional group in question, i.e. a homologous series of alkylbenzenes for methylene selectivity. The natural logarithm of the capacity factor is then plotted versus the unit number of the functional group for each homolog; the slope of the resultant line is the natural logarithm of the group selectivity, $\ln \alpha$. Figure 5-1 illustrates such a plot of methylene selectivity on column DMAP 3 for the homologous alkylbenzene series of toluene through pentylbenzene. The slope of the plot, which is $\ln \alpha_{\text{methylene}}$, is 0.6773; the resultant $\alpha_{\text{methylene}}$ value is then 1.969.

Antle and Snyder (1984) and Antle et al. (1985) state that there are two different types of RP column selectivity, namely solvophobic and chemical. Solvophobic selectivity arises from hydrophobic interactions between the solute molecules and the stationary phase. Chemical selectivity comes about from strong interactions (for example, hydrogen bonding or complexation) between the solute molecules and specific active sites such as silanol groups or trace metal contaminants on the silica surface (Antle and Snyder, 1984; Jandera, 1986). A third type of selectivity, shape selectivity, can also be exhibited by chemically bonded phases. Since these phases consist of lengthy alkyl chains bonded to the silica surface, the conformation of the bonded chains can play an important role in retention, especially for large molecules. When these chains are well solvated by the mobile phase, such as when the mobile phase has a large

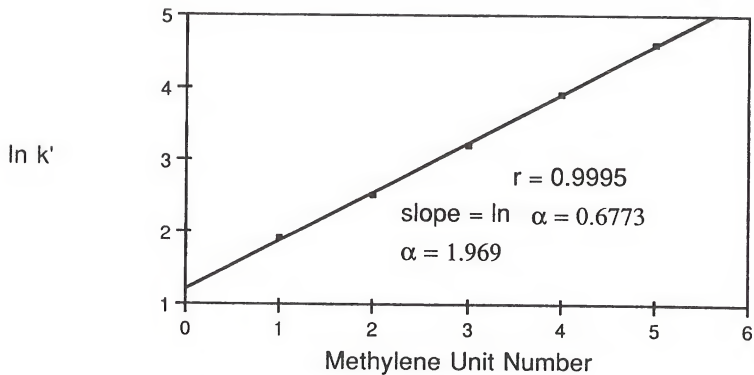


Figure 5-1. Methylene selectivity plot for silica column DMAP3.
 The slope of the plot is $\ln \alpha_{\text{methylene}}$.

proportion of organic modifier, the chains become more fully extended and shape selectivity is increased (Martire and Boehm, 1983). Wise and Sander (1985) refer to this as the "slot model." They have postulated that when the closely packed bonded RP chains are extended, the stationary phase surface can be visualized as containing a number of long narrow "slots" between these extended chains into which solute molecules can penetrate. Since planar and/or linear molecules can more deeply penetrate these slots and therefore interact more strongly with the stationary phase, they are preferentially retained over nonplanar and/or nonlinear molecules.

Examination of chromatographic selectivity can be very useful in studies of retention mechanisms in LC. As discussed in Chapter IV, the capacity factor, k' , is the most widely studied chromatographic parameter, since it is a normalized measure of solute retention. However, the capacity factor is directly proportional to the volume phase ratio (stationary/mobile) of the chromatographic column. The phase ratio is dependent upon bonded group chain length, alkyl bonding density, the pore structure of the silica support and the homogeneity of the packing bed of the column. Therefore, when comparing intercolumn capacity factors there are many variables to consider, making it very difficult to draw conclusions about intercolumn retention behavior. Selectivity values for solutes are not affected by the phase ratio of the chromatographic column, since they

are measurements of retention differences (i.e. ratios) rather than absolute measures of retention. Therefore intercolumn selectivity differences are not due to different column phase ratios, but rather are due to actual differences in the structure of the bonded alkyl chains in the different columns. In general, selectivity values for a particular type of bonded phase are independent of the specific column used (Antle and Snyder, 1984; Colin et al., 1983a and 1983b; Krstulovic et al., 1983; Melander and Horvath, 1982), implying that very fundamental aspects of the retention process are reflected by selectivity behavior.

Examination of methylene selectivity offers an additional advantage for retention mechanism studies. Since methylene selectivity is solely due to solvophobic selectivity, it is quite insensitive to the presence of residual silanol groups on the bonded phase surface. For solutes which have highly polar and/or hydrogen bonding functional groups, the presence of these silanol groups can lead to poor chromatographic peak shape as well as anomalous retention due to chemical selectivity. However, methylene selectivity values for a homologous series of such compounds will be largely unaffected by these specific interactions, even though retention of individual members of the series may be susceptible to these chemical interactions (Johnson, 1986).

In the present work, chromatographic selectivity was examined as a function of stationary phase alkyl bonding

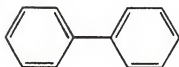
density. When the same mobile phase composition is utilized in comparing different stationary phase selectivities, mobile phase contributions to the free energy of transfer should be equivalent. Under such conditions, changes in selectivity are attributable to differences in the stationary phase structure (Lochmuller et al., 1985). Methylene selectivity and phenyl selectivity were examined on octadecyl silica and CPG reversed phases. Methylene selectivity was examined using the alkylbenzenes as test solutes; phenyl selectivity was probed with the phenyl homologous series consisting of benzene, biphenyl and p-terphenyl, whose structures are shown in Figure 5-2. National Bureau of Standards (NBS) column evaluation test mixture 1 (PAH) was also used to measure overall polycyclic aromatic hydrocarbon (PAH) selectivity; this mixture contains benzo[a]pyrene (BaP), 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN) and phenanthro[3,4-c]-phenanthrene (PhPh), whose structures are shown in Figure 5-3.

Experimental Procedure

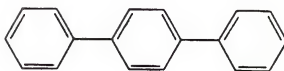
The liquid chromatographic system, silica and CPG columns and solvents used for the selectivity measurements are described in Chapter IV. Toluene (Eastman Organic Chemicals, Rochester, NY), ethylbenzene (Fisher Scientific, Fairlawn, NJ), propylbenzene (Alfa Products, Danvers, MA), butylbenzene (Eastman) and pentylbenzene (Alfa) standards were made up in HPLC grade methanol for methylene



Benzene

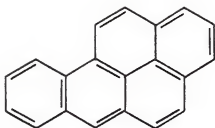


Biphenyl



p-Terphenyl

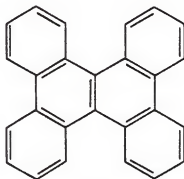
Figure 5-2. Structures of phenyl selectivity test solutes.



Benzo[a]pyrene (BaP)



Phenanthro [3,4-c] phenanthrene (PhPh)



1,2:3,4:5,6:7,8-Tetrabenzonaphthalene (TBN)

Figure 5-3. Structures of National Bureau of Standards (NBS) column evaluation test mixture number 1 (PAH) solutes.

selectivity studies. Benzene (Mallinckrodt, Inc., Paris, KY), biphenyl (Eastman, recrystallized three times from ethanol) and p-terphenyl (Sigma Chemical Co., St. Louis, MO) methanolic standards comprised the phenyl selectivity test solutes. The NBS (Gaithersburg, MD) column evaluation test mixture was kindly supplied by Dr. Lane Sander. Methylene and phenyl selectivity studies were conducted at 35.0 °C with a 55/45 methanol/water mobile phase on the silica columns; these studies were also performed with a 85/15 acetonitrile/water mobile phase at 35.0 °C for both the silica and CPG columns. The NBS test mixture was also evaluated on the silica and CPG columns with a 85/15 acetonitrile/water mobile phase but at ambient temperature.

Results and Conclusions

Silica-Based Stationary Phases

Methylene and phenyl selectivities as a function of octadecyl bonding density for the 55/45 methanol/water and 85/15 acetonitrile/water mobile phase systems are tabulated in Tables 5-1 and 5-2. Since the selectivity values are calculated from the slopes of plots of $\ln k'$ versus homolog unit number for each stationary phase, the least squares linear regression coefficients of correlation for each of these plots are included to verify that linear behavior is being followed. Colin et al. (1983a) state that a linear relationship exists between $\ln k'$ and the homolog unit number for unit numbers above three to five. This number of units is termed the critical carbon number and it results

Table 5-1. Methylene and phenyl selectivities at 35.0 °C as a function of silica octadecyl bonding density for 55/45 methanol/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	Methylene Selectivity	Methylene ¹ Correlation Coefficient	Phenyl Selectivity	Phenyl ² Correlation Coefficient
1.60	1.721	0.9989	5.479	0.9998
1.74	1.916	0.9993	7.272	0.9997
1.98	1.959	0.9997	7.053	1.0000
2.07	1.925	0.9994	7.385	0.9996
2.09	1.967	0.9999	7.196	1.0000
2.75	1.936	0.9993	7.606	0.9999
3.06	1.986	0.9996	7.830	0.9999
3.24	1.969	0.9995	7.941	0.9999
3.34	1.968	0.9994	8.134	0.9997
3.56	1.927	0.9994	7.965	0.9998
3.60	1.963	0.9997	8.170	0.9997

¹ Coefficient of correlation for the plot of $\ln k'$ versus carbon number; slope of this line is $\ln(\text{methylene selectivity})$.

² Coefficient of correlation for the plot of $\ln k'$ versus phenyl number; slope of this line is $\ln(\text{phenyl selectivity})$.

Table 5-2. Methylene and phenyl selectivities at 35.0 °C as a function of silica octadecyl bonding density for 85/15 acetonitrile/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	Methylene Selectivity	Methylene ¹ Correlation Coefficient	Phenyl Selectivity	Phenyl ² Correlation Coefficient
1.60	1.134	0.9971	1.423	0.9984
1.74	1.291	0.9991	1.901	0.9996
1.98	1.275	0.9993	1.924	0.9994
2.07	1.214	0.9935	1.944	1.0000
2.09	1.308	0.9994	1.950	0.9999
2.75	1.530	0.9906	2.000	0.9995
2.84	1.352	0.9983	2.042	0.9999
3.06	1.339	0.9988	2.039	0.9997
3.15	1.344	0.9974	2.011	0.9995
3.24	1.348	0.9990	2.033	0.9992
3.34	1.364	0.9943	2.016	0.9992
3.56	1.350	0.9995	2.031	0.9995
3.60	1.358	0.9999	2.111	0.9997

¹ Coefficient of correlation for the plot of $\ln k'$ versus carbon number; slope of this line is $\ln(\text{methylene selectivity})$.

² Coefficient of correlation for the plot of $\ln k'$ versus phenyl number; slope of this line is $\ln(\text{phenyl selectivity})$.

from the fact that the effect of an additional homolog unit should only become constant when it is sufficiently removed from the basic functional group. Thus for homologs below the critical carbon number, the plot of $\ln k'$ versus homolog unit number is expected to exhibit curvature. However, this departure from linearity is generally small for RPLC systems, causing a very limited influence on the average slope of the plot (Colin et al., 1983a). This expected curvature was not found for either mobile phase system, as all of the correlation coefficients are greater than or equal to 0.991.

Methylene selectivity versus octadecyl bonding density is plotted in Figure 5-4 for the 55/45 methanol/water system and in Figure 5-5 for the 85/15 acetonitrile/water system for all of the silica stationary phases except for the lowest bonding density phase ($1.60 \mu\text{mol}/\text{m}^2$). This bonded phase can be omitted from both of the silica methylene selectivity plots because its selectivity in both cases can be shown to be an outlier based on the Q-test at the 99% confidence level for the 55/45 methanol/water mobile phase and at the 90% level for the 85/15 acetonitrile/water mobile phase data (Peters et al., 1974). The average methylene selectivity value \pm one standard deviation for the remaining ten bonded phases with the methanol/water mobile phase is 1.952 ± 0.024 ; for the remaining twelve stationary phases in the acetonitrile/water system this value is 1.339 ± 0.074 . Using 55/45 methanol/water mobile phase systems and reversed

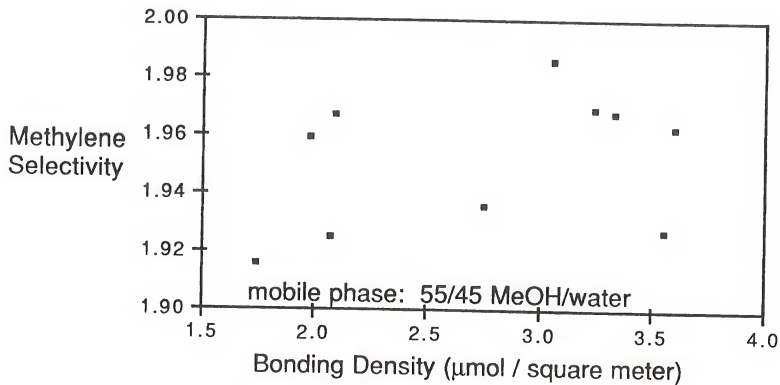


Figure 5-4. Plot of methylene selectivity versus octadecyl bonding density for silica-based columns at 35.0 °C for 55/45 methanol/water mobile phase.

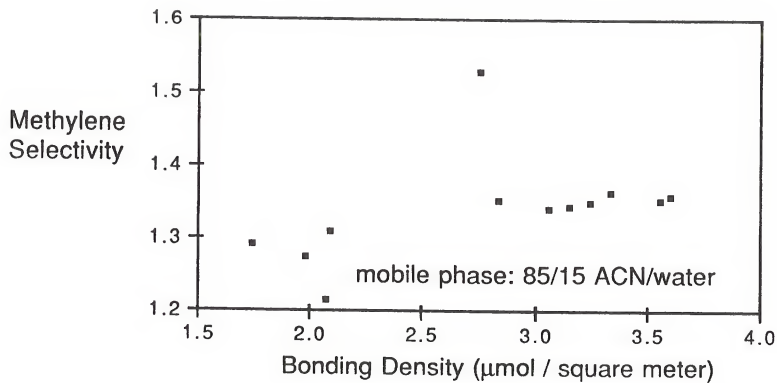


Figure 5-5. Plot of methylene selectivity versus octadecyl bonding density for silica-based columns at 35.0 °C for 85/15 acetonitrile/water mobile phase.

phase octadecyl columns, Colin et al. (1983a) and Karger et al. (1976) report methylene selectivity values of 2.14 and 2.0 respectively. For octadecyl silica columns and 85/15 acetonitrile/water mobile phases Colin et al. (1983a), Karger et al. (1976) and Krstulovic et al. (1983) report methylene selectivity values of 1.40, 1.3 and 1.4 respectively; therefore our reported methylene selectivity values are comparable to literature values in both mobile phase systems. It is not surprising that these methylene selectivities are approximately constant in either system since methylene selectivity is a type of solvophobic selectivity, coming about solely from hydrophobic interactions between the solute molecules and the stationary phase. It was expected that such a nonspecific interaction would be unaffected by the greater chain ordering resulting from increasing octadecyl bonding density. The differences in methylene selectivity values for the two mobile phase systems is due to differences in bonded chain solvation. Acetonitrile is able to better solvate the hydrocarbonaceous bonded chains and therefore results in a more robust solvation layer than methanol does. Since methylene selectivity is a measure of the hydrophobic interactions between a methylene group and the stationary phase, the methylene selectivity value for the acetonitrile system is smaller than that for the methanol system because there is less difference in hydrophobicity between a methylene group and the solvated stationary phase structure in the

acetonitrile system. Put another way, the acetonitrile/bonded phase interphase is more nonpolar than the methanolic one; therefore a methylene group will experience less hydrophobic interactions in the acetonitrile system, resulting in a lower methylene selectivity value (Karger et al., 1976).

Examination of the relationship between phenyl selectivity and bonding density is facilitated by inspection of Figures 5-6 and 5-7 for the 55/45 methanol/water and 85/15 acetonitrile/water mobile phase systems respectively. The plots show that phenyl selectivity increases with increasing octadecyl bonding density in an approximately linear fashion with least squares linear regression slopes of 0.547 and 0.0835 and coefficients of correlation of 0.956 and 0.917 for the methanol/water and acetonitrile/water systems respectively. This correlation between phenyl selectivity and octadecyl bonding density can be attributed to shape selectivity. As previously mentioned, other workers have noted that chromatographic selectivity is affected by the shape of the solute molecules (Lochmuller et al., 1985; Martire and Boehm, 1983; Tanaka et al., 1982). They have predicted that solute selectivity should decrease as a function of solute shape in the order rodlike > planar > chainlike. It has also been suggested that selectivity of rodlike or rigid solutes increases with increasing bonded chain surface coverage (Engelhardt et al., 1982; Hemetsberger et al., 1979; Wise et al., 1981). This effect

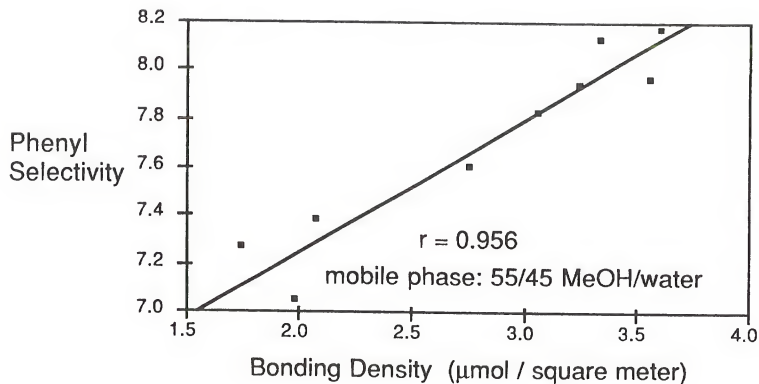


Figure 5-6. Plot of phenyl selectivity versus octadecyl bonding density for silica-based columns at 35.0 °C for 55/45 methanol/water mobile phase.

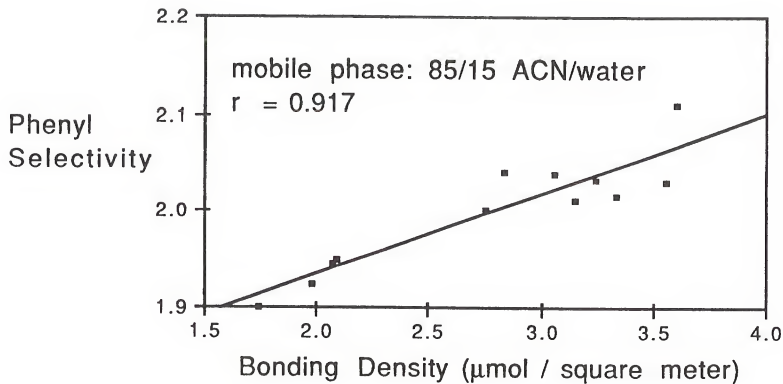


Figure 5-7. Plot of phenyl selectivity versus octadecyl bonding density for silica-based columns at 35.0 °C for 85/15 acetonitrile/water mobile phase.

has been explained in terms of increased ordering of the bonded RP chains (Krstulovic et al., 1983; Lochmuller et al., 1985; Martire and Boehm, 1983).

Sander and Wise (1984a and 1984b; Wise and Sander, 1985) and Wise and May (1983) have extensively examined the effect of alkyl bonding density on retention and selectivity for polycyclic aromatic hydrocarbons (PAH). They have studied the PAH selectivity of monomeric and polymeric octadecyl phases with bonding density ranges of 1.8 to 3.2 $\mu\text{mol}/\text{m}^2$ and 2.7 to 7.3 $\mu\text{mol}/\text{m}^2$ respectively. Their studies indicate that the polymeric phases exhibit much greater PAH selectivity than the monomeric ones and that the polymeric phase PAH selectivity increases with increasing bonded phase surface coverage. They initially attributed this behavior to some fundamental difference in the structures of the monomeric and polymeric bonded phases. However, Verzele and Mussche (1983) concluded that there is no true difference in the nature of polymeric and monomeric bonded phases, and that their differences in chromatographic behavior are attributable to differences in surface coverage. In later comparisons of monomeric and polymeric bonded phases of varying bonded alkyl chain length, Sander and Wise (1987) concluded that the changes in PAH selectivity that they had earlier observed were not necessarily due to fundamental differences in the two phases but rather could be attributed to changes in the overall stationary phase thickness.

Wise and Sander's (1985) "slot model" was postulated based on their PAH selectivity studies. They found that for polymeric phases with high bonding densities (greater than about $5.1 \mu\text{mol/m}^2$) nonplanar solutes eluted before planar ones and that nonlinear solutes eluted before linear ones, even if the solutes compared had similar molecular weight, overall shape and molecular dimensions. Additionally, they found that selectivity between planar/nonplanar and linear/nonlinear PAHs increases with the degree of nonplanarity and nonlinearity. Their "slot model" postulates that nonplanar solutes have a greater "thickness", hindering penetration of the solute into the narrow slots between the bonded alkyl chains. If both wide and narrow slots exist in the stationary phase structure due to inhomogeneous distribution of the bonded chains on the surface, retention will be greatest for long narrow solutes, since they would fit into more available slots than thicker "square" shaped molecules. The situation is analogous for linear molecules, which would show greater retention than nonlinear ones. This also corresponds with Martire and Boehm's "unified theory of retention and selectivity in liquid chromatography" (1983) which predicts that shape selectivity is greater for rigid rod solutes than for globular solutes, especially when the stationary phase chains are fully extended or more rigid. Sander and Wise (1985) argue that higher alkyl density polymeric phases are more extended and rigid than low density polymeric or monomeric ones.

Sander and Wise (1984a) have devised a simple empirical LC test to gauge the relative monomeric or polymeric nature of a bonded phase. They found that the elution order of a three component PAH test mixture of phenanthro[3,4-c]-phenanthrene (PhPh), 1,2:3,4:5,6:7,8-tetrabutyl naphthalene (TBN) and benzo[a]pyrene (BaP) at ambient temperature with a 85/15 acetonitrile/water mobile phase is dependent on the type of phase and the surface coverage. For monomeric phases (bonding densities up to about $3.2 \mu\text{mol}/\text{m}^2$) the elution order is $\text{BaP} \leq \text{PhPh} < \text{TBN}$; for oligomeric phases (bonding densities of 3.3 to about $4.2 \mu\text{mol}/\text{m}^2$) the elution order is $\text{PhPh} < \text{BaP} < \text{TBN}$. Polymeric phases (bonding density $> 4.3 \mu\text{mol}/\text{m}^2$) give the elution order $\text{PhPh} < \text{TBN} < \text{BaP}$. Monomeric phases were synthesized with the monochlorosilane; oligomeric and polymeric phases were prepared using the trichlorosilane reagent. Each type of phase also results in a different narrow range of values for TBN/BaP selectivity. By examining the elution order of the compounds in the test mixture, PAH selectivity of any RP column can be quickly predicted.

The retention behavior of these compounds can be attributed to their shapes. Phenanthro[3,4-c]phenanthrene and TBN are nonplanar, due to steric hindrance of neighboring aromatic rings. Of the two, PhPh is the more nonplanar, exhibiting a helical shape, while TBN is described as saddle shaped. Although TBN and PhPh are six ring PAHs, BaP is a five ring PAH and is completely planar;

therefore as just discussed it exhibits greater retention as the polymeric character of the bonded phase (and its bonding density) is increased (Sander and Wise, 1984a).

In light of what has just been discussed, the trend of greater phenyl selectivity with increasing octadecyl bonding density shown by our work is not surprising. These results correlate well with Sander and Wise's (1985) "slot model", since with increasing bonding density the "slots" between the extended octadecyl chains become increasingly long and narrow. Therefore selectivity is expected to increase for planar, linear solutes such as the biphenyl and p-terphenyl used to measure phenyl selectivity in this work. However, a more rigorous explanation for the correlation between phenyl selectivity and bonding density can be offered in light of the Dill (1987a) interphase stationary phase model discussed in Chapter I. As alkyl surface densities increase, the corresponding configurational constraints are also increased, creating a more rigid and ordered chain packing structure. In this model, the driving force for retention is the creation of a solute-sized cavity in the stationary phase chain packing structure. As bonding density and consequently chain ordering are increased the energy required for cavity formation also increases. Creation of a narrow linear cavity for solute retention will require less energy than that required to create a wider cavity such as is needed for nonlinear or nonplanar solute retention. Therefore selectivity for these linear and planar molecules

will increase with alkyl bonding density, as predicted by this theory and as borne out by our experimental results.

It is interesting to compare the slope of the 85/15 acetonitrile/water phenyl selectivity plot (0.0835) to that for the 55/45 methanol/water plot (0.547). This disparity can probably be attributed to the different structures of the solvation layers on the bonded phase surfaces in the two very different mobile phase systems. The 85/15 acetonitrile/water solvation layer is relatively robust; at any of the bonded phase alkyl densities the stationary phase surface will be well solvated and the chains well extended. This means that relative retention will only be affected to a small extent by changes in bonding density; chain ordering will increase very little with increased packing constraints because the chains are already well extended and relatively ordered. In 55/45 methanol/water the chains are not well solvated and are in a relatively collapsed configuration; they are rather disordered. As the bonding density increases the chains become increasingly ordered as well as being much more extended. Thus shape selectivity will be affected by bonding density to a much greater extent in the methanol/water system; this is exhibited by the larger slope of the phenyl selectivity plot.

The selectivity behavior of Sander and Wise's PAH test mixture on these silica columns, compiled in Table 5-3, further confirms that shape selectivity increases with increasing alkyl bonding density. For bonding densities of

Table 5-3. Tetrabutyl-naphthalene(TBN)/benzo[a]pyrene(BaP) selectivity as a function of silica octadecyl bonding density for 85/15 acetonitrile/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	TBN/BaP ¹ Selectivity	Stationary ² Phase Behavior	Temperature (°C)
1.60	1.60	monomeric	25.0
1.74	1.68	monomeric	24.0
1.98	1.68	monomeric	26.0
2.07	1.72	monomeric	26.0
2.09	1.70	monomeric	26.0
2.75	1.73	monomeric	25.5
2.84	1.72	monomeric	25.5
3.06	1.75	monomeric	25.0
3.15	1.73	monomeric	25.0
3.24	1.72	monomeric	27.0
3.34	1.70	monomeric	29.0
3.56	1.69	monomeric	26.0
3.60	1.56	oligomeric	26.0

¹ Ratio of k'_{TBN} to k'_{BaP} .

² Stationary phase characterization based on classification system of Sander and Wise (1984a). If solute elution order is $\text{BaP} < \text{PhPh} < \text{TBN}$, stationary phase is considered to be monomeric; elution order of $\text{PhPh} < \text{BaP} < \text{TBN}$ is considered to be oligomeric.

1.74 to $3.56 \mu\text{mol}/\text{m}^2$ the TBN/BaP selectivity is about 1.7 and the elution order is BaP = PhPh < TBN. At $3.60 \mu\text{mol}/\text{m}^2$, the elution order changes to PhPh < BaP < TBN and the TBN/BaP selectivity is 1.56. The planar BaP molecule is now retained longer than the helical PhPh. Although this is classified as "oligomeric" type behavior by Sander and Wise (1984a), this stationary phase was prepared using the monochlorosilane as opposed to the trichlorosilane reagent used by Sander and Wise to prepare the oligomeric bonded phases. The oligomeric bonded phases are actually polymeric type phases whose bonding density (or "thickness") has been controlled by sequential polymerization. The fact that our monomeric phase exhibits the same PAH selectivity as Sander and Wise's polymeric phases indicates that PAH selectivity and shape selectivity are probably not a function of the degree of stationary phase polymerization but rather are a function of alkyl bonding density. The correlation of phenyl selectivity with alkyl bonding density further supports this conclusion.

Controlled Pore Glass-Based Stationary Phases

Table 5-4 is a compilation of methylene and phenyl selectivities of the controlled pore glass (CPG) octadecyl bonded phases versus their octadecyl bonding density for the 85/15 acetonitrile/water mobile phase; this data is also plotted in Figures 5-8 and 5-9. Although very scattered, the methylene selectivity seems to be approximately constant, as in the case of the silica stationary phases.

The average methylene selectivity \pm one standard deviation is 1.210 ± 0.031 . The argument for constant methylene selectivity for the silica bonded phases can similarly be applied to these CPG phases.

Phenyl selectivity for these CPG phases does not show the linear trend exhibited by the silica bonded phases. The phenyl selectivities are quite scattered and do not seem to correlate with bonding density in any manner. Therefore, no conclusions can be drawn about the effect of bonding density on phenyl selectivity for the CPG phases. Selectivity results from the NBS PAH test mixture are listed in Table 5-5. These phases exhibit monomeric behavior at all of the bonding densities examined; the TBN/BaP selectivity values range from 1.66 to 1.80 and are more scattered than those for the silica. In summary, our CPG selectivity data is inconclusive, especially when compared to the silica data. Further experiments on this support are warranted if its selectivity trends are to be determined.

Table 5-4. Methylene and phenyl selectivities at 35.0 °C as a function of controlled pore glass octadecyl bonding density for 85/15 acetonitrile/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	Methylene Selectivity	Methylene ¹ Correlation Coefficient	Phenyl Selectivity	Phenyl ² Correlation Coefficient
1.70	1.174	0.9874	1.489	0.9902
2.59	1.199	0.9928	1.636	0.9961
2.68	1.252	0.9980	1.719	0.9975
2.72	1.222	0.9995	1.557	0.9983
2.83	1.210	0.9937	1.678	0.9971
3.21	1.240	0.9976	1.748	0.9966
3.30	1.171	0.9879	1.577	0.9980

¹ Coefficient of correlation for the plot of $\ln k'$ versus carbon number; slope of this line is $\ln(\text{methylene selectivity})$.

² Coefficient of correlation for the plot of $\ln k'$ versus phenyl number; slope of this line is $\ln(\text{phenyl selectivity})$.

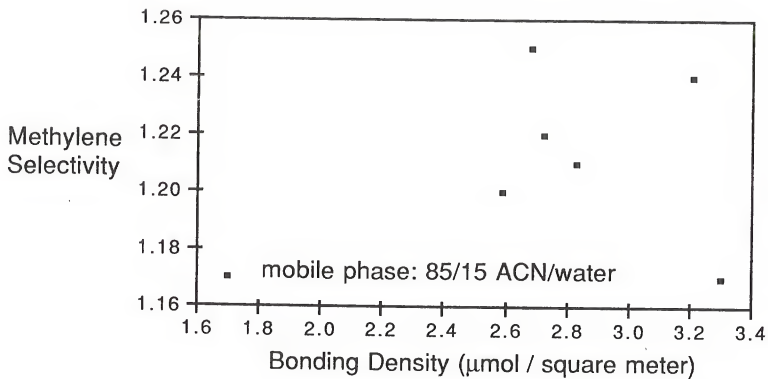


Figure 5-8. Plot of methylene selectivity versus octadecyl bonding density for CPG-86-based columns at 35.0 °C for 85/15 acetonitrile/water mobile phase.

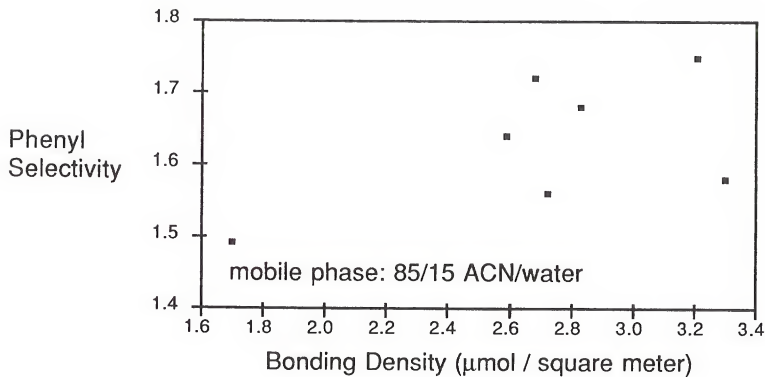


Figure 5-9. Plot of phenyl selectivity versus octadecyl bonding density for CPG-86-based columns at 35.0 °C for 85/15 acetonitrile/water mobile phase.

Table 5-5. Tetrabutyl naphthalene (TBN)/benzo[a]pyrene (BaP) selectivity as a function of controlled pore glass octadecyl bonding density for 85/15 acetonitrile/water mobile phase.

C ₁₈ Bonding Density ($\mu\text{mol}/\text{m}^2$)	TBN/BaP ¹ Selectivity	Stationary ² Phase Behavior	Temperature (°C)
1.70	1.66	monomeric	24.5
2.59	1.79	monomeric	25.0
2.68	1.74	monomeric	26.0
2.72	1.71	monomeric	25.0
2.83	1.79	monomeric	25.0
3.21	1.80	monomeric	25.5
3.30	1.72	monomeric	25.5

¹ Ratio of k'_{TBN} to k'_{BaP} .

² Stationary phase characterization based on classification system of Sander and Wise (1984a). If solute elution order is BaP < PhPh < TBN, stationary phase is considered to be monomeric; elution order of PhPh < BaP < TBN is considered to be oligomeric.

CHAPTER VI CONCLUSIONS

Syntheses of RP Stationary Phases

The results presented in Chapter II show that the use of ultrasound as a driving force for the synthesis of reversed phase (RP) stationary phases is a viable alternative to traditional reflux methods. This especially has advantages on an industrial-sized scale; the use of ultrasonification at ambient temperatures results in stationary phases of comparable bonding density ($3.35 \mu\text{mol}/\text{m}^2$) to those produced under refluxed conditions ($3.44 \mu\text{mol}/\text{m}^2$), with considerably less power consumed and therefore at a reduced cost. Another advantage of ultrasonification over reflux in a manufacturing situation is with respect to Occupational Safety and Health Administration (OSHA) guidelines; the use of reflux requires adherence to very stringent OSHA fire codes, resulting in additional manufacturing expenses. Ultrasound reactions at subambient temperature (i.e. 3.0°C) produce stationary phases with very high alkyl bonding densities ($3.60 \mu\text{mol}/\text{m}^2$). Although our reason for pursuing the synthesis of high density reversed phases was to examine their retention and selectivity characteristics, there are practical advantages to these phases as well. In order to separate

two compounds with very similar free energies of retention (and therefore very similar retention times) their chromatographic resolution, R_S , must be maximized. Since

$$R_S = ((N^{1/2})/4) ((\alpha - 1)/\alpha) (k'/(k'+1)),$$

where N is the number of theoretical plates (column efficiency), α is the selectivity factor for the two compounds and k' is the capacity factor of the longer retained compound, an increase in α will increase R_S , resulting in an improved separation of the two compounds. Increasing selectivity is the optimum method for increasing chromatographic resolution without increasing the analysis time. As we have shown in our experimental results and discussions in Chapter V, one method of increasing α is to increase the alkyl bonding density. Therefore, these high density phases offer a practical solution for the resolution of difficult to separate compounds.

High alkyl density stationary phases are also more amenable to alkaline pH conditions than are low density phases. Silica-based bonded phases are usually only practical in the pH range of 2.5 to 7.5 since at pH lower than 2.5 the Si-C bond is cleaved and at pH higher than 7.5 appreciable dissolution of the siliceous support occurs (Melander and Horvath, 1980). High density bonded phases can offer a better "protection" of the siliceous support from the mobile phase since by virtue of the closely spaced alkyl groups less of the mobile phase is able to reach the silica surface. Preliminary longevity studies of an

ultrasound synthesized stationary phase with a bonding density of $3.35 \mu\text{mol}/\text{m}^2$ have shown impressive ruggedness-- chromatographic performance did not appreciably degrade in terms of efficiency or selectivity until 6000 ml of 30/30/40 methanol/acetonitrile/pH 11 buffer had been passed through the column (Novak, 1987). It can be concluded that these high density phases show great promise for extending the viable working pH range in reversed phase systems. Buszewski et al. (1986) have prepared monomeric octadecyl stationary phases of various bonding densities; they found that the high density phases resulted in better resolution of purine compounds than the lower density phases because the higher alkyl surface coverage shields residual silanol groups on the silica surface, resulting in a marked decrease in peak tailing. High alkyl density phases should then be well suited for the improved separation of other types of basic compounds. In summary, high alkyl density reversed phase packings offer many practical advantages over lower density phases.

For both silica and controlled pore glass (CPG) bonded phase syntheses, 4-dimethylaminopyridine (4-DMAP) proved to be a much more effective acid-acceptor catalyst than 2,6-lutidine. As with silica, ultrasound CPG reactions resulted in bonding densities comparable to those achieved under traditional refluxed conditions. However, at any of the octadecyl reaction conditions, the silica substrate had consistently higher reactivity than the CPG, resulting in

higher silica bonding densities. Contrary to our expectations, the smaller pore diameter CPG (86 Angstroms) was more reactive in the octadecyl reaction than the 167 Angstrom CPG. Both of these trends were reversed in the trimethylsilane bonding reaction. Except for the higher B_2O_3 content of the CPG material, we have been unable to postulate a reason for the seemingly anomalous CPG results.

Validity of Chromatographic Partition Coefficient Measurements

Experimental

In order to discuss the implications of the behavior of chromatographic partition coefficients as a function of octadecyl bonding density, the validity of the chromatographic partition coefficient (K) measurement should first be established. Recall that $K = k'/(V_S/V_m)$. Measurement of k' , the solute capacity factor is defined as $(V_r - V_m)/V_m$ where V_r is the solute retention volume and V_m is the mobile phase void volume. The problems involved in the measurement of V_m and V_S have been discussed in Chapter IV, as has our rationale for our choices of conventions for their measurement. In order to test the validity of the chromatographic partition coefficient measurement, we decided to perform an independent dynamic measurement of the partition coefficient. A known mass of a chromatographic test solute (benzene or naphthalene) was placed in a stirred volumetric flask containing a known mass of stationary phase DMAP5 ($3.60 \mu\text{mol/m}^2$) and a known mass of mobile phase (55/45 methanol/water or 85/15 acetonitrile/water). The stationary

phase volume (V_s) was calculated using equation 4-4; V_m was calculated by dividing the mobile phase mass by its density, which had been measured at ambient temperature. The volumetric flask was sealed and stirred continuously over a 48 hour period. Ten microliter samples of the mobile phase portion of the mixture were taken in quadruplicate while stirring continued at 16, 20, 32 and 48 hour intervals from when the sample was added to the system. These samples were injected into the HPLC system (described in Chapter IV) at 35.0 °C consisting of column DMAP5 and the appropriate methanol or acetonitrile mobile phase system and the sample responses (peak heights) were measured. The amount of test solute in the sample was determined by comparison of the sample response to a calibration plot prepared for standard solutions of the solute under the same chromatographic conditions. For benzene in 55/45 methanol/water, the total range of the calibration plot was from 1297 to 0.1297 μg benzene; the log-log calibration plot is shown in Figure 6-1. For naphthalene in 85/15 acetonitrile/water, the total range of the calibration plot was from 100.0 to 0.01000 μg and its corresponding log-log plot is shown in Figure 6-2. Once the sample response of the unknown was measured, the calibration standards bracketing the unknown were diluted and their responses measured in order to prepare a more precisely determined calibration plot in the concentration range of the unknown. The benzene and naphthalene calibration plots are shown respectively in Figures 6-3 and

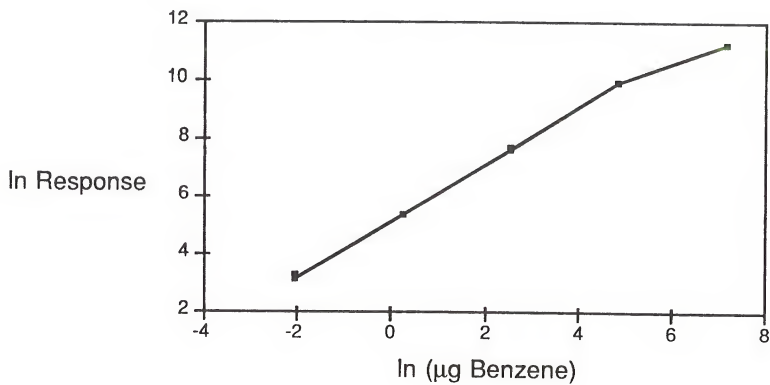


Figure 6-1. Log-log calibration plot for benzene on column DMAP5 at 35.0 °C for 55/45 methanol/water mobile phase.

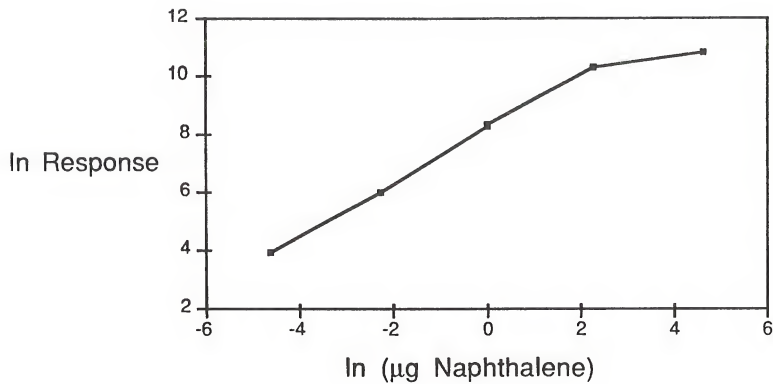


Figure 6-2. Log-log calibration plot for naphthalene on column DMAP5 at 35.0 °C for 85/15 acetonitrile/water mobile phase.

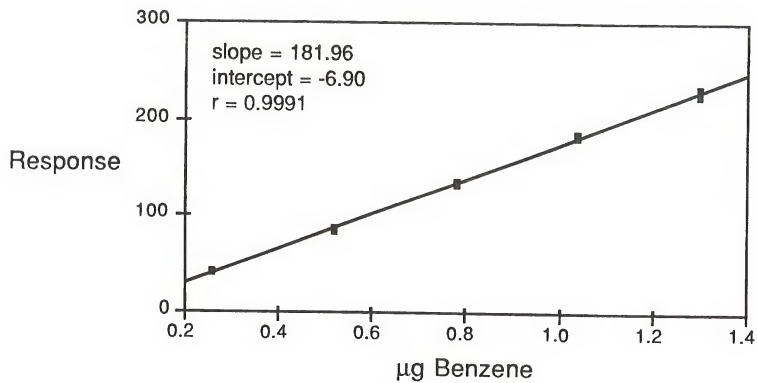


Figure 6-3. Calibration plot for benzene on column DMAP5 at 35.0 °C for 55/45 methanol/water mobile phase.

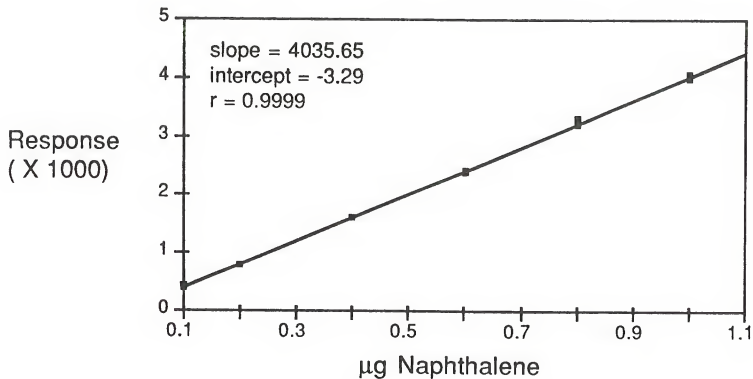


Figure 6-4. Calibration plot for naphthalene on column DMAP5 at 35.0 °C for 85/15 acetonitrile/water mobile phase.

6-4. All calibration standards were injected at least in quadruplicate.

Precautions were taken in order to ensure an accurate dynamic measurement of the partition coefficient. The initial calibration plots were prepared over a large concentration range in order to find the solute concentration at the saturation point of the stationary phase at which the column is overloaded, resulting in nonlinear response. The amount of solute that was added to the volumetric flask was calculated so that upon dilution the solute concentration would be within the linear range of the calibration plot. The solute was added to and sampled from the volumetric flask under continuous stirring so as to lessen the probability of solute adsorption on the flask walls and on the stationary phase surface. The detector responses for replicate injections at each calibration level were all used in preparing the calibration plot rather than using the average response at each level; this ensures that an outlier response will have a negligible effect on the overall slope and intercept of the plot. The volumetric flask contents were sampled at four different time intervals to ensure that complete equilibration of the solute between the mobile and stationary phases had occurred.

Results and Conclusions

The individual responses of the 16 solute samples taken from the volumetric flasks at different sampling times were each within one standard deviation of the average response

of the 16 injections; therefore equilibrium distribution of the sample between the stationary and mobile phases was complete at the first sampling interval (16 hours). For the benzene solute in 55/45 methanol/water the least squares linear regression equation of the calibration plot was: Response = (181.96 response units/ μg benzene) (μg benzene) - 6.90 with a coefficient of correlation of 0.9991. The average response (\pm one standard deviation) for the dynamic benzene sample was 140.09 ± 5.49 . This average response corresponded to 0.8078 μg benzene in the equilibrium mobile phase samples injected; the benzene amount range (calculated from the average response plus and minus one standard deviation) was from 0.8380 to 0.7777 μg benzene. The amount of benzene that partitioned into the stationary phase was calculated as the difference between the original amount of benzene added to the flask (corrected for mobile phase dilution) and the equilibrium amount in the mobile phase. The calculation of the dynamic partition coefficient is:

$$K = \frac{\text{equilibrium } \mu\text{g benzene in stationary phase}/V_s}{\text{equilibrium } \mu\text{g benzene in mobile phase}/V_m}$$

where V_s and V_m are the appropriate volumes for the volumetric flask contents. The average dynamic partition coefficient for benzene between the 55/45 methanol/water mobile phase and the DMAP5 stationary phase was 8.52; when the benzene concentration range for the measurement was considered, the dynamic equilibrium partition coefficient ranged from 5.97 to 11.25.

For the naphthalene solute in 85/15 acetonitrile/water the least squares linear regression calibration plot equation was:

Response = (4035.65 response units/ μg naphthalene) \times (μg naphthalene) - 3.29 with a coefficient of correlation of 0.9999. The average response of the dynamic naphthalene sample \pm one standard deviation was 430.87 ± 13.89 . This response correlated to an equilibrium amount of naphthalene in the mobile phase of $0.1076 \mu\text{g}$ with a range of $0.1110 \mu\text{g}$ to $0.1041 \mu\text{g}$ when \pm one standard deviation was considered. The average dynamic partition coefficient for naphthalene between 85/15 acetonitrile/water mobile phase and DMAP5 stationary phase was 0.041; the high end of the naphthalene range could not be used since the amount of naphthalene it corresponded to ($0.1110 \mu\text{g}$) was larger than the original amount put into the volumetric flask, once dilution was accounted for ($0.1077 \mu\text{g}$). When the low end of the naphthalene concentration range was considered the dynamic partition coefficient was 1.51.

When chromatographic partition coefficients are compared to those obtained in the dynamic equilibrium experiment, the chromatographic K is consistently higher. For the benzene system, $K_{\text{chromatographic}} = 17.98$ and $K_{\text{dynamic}} = 8.51$; for the acetonitrile system $K_{\text{chromatographic}} = 4.41$ and $K_{\text{dynamic}} = 0.041$. There are a number of reasons for these discrepancies. When the dilution factor for the amount of solute added to the

volumetric flask was determined, it was based solely on the volume of mobile phase in the flask; the volume of the stationary phase was not considered since the density of the derivatized silica was unknown. When the contents of the stirred flask were sampled, it was impossible to only draw up mobile phase for sampling; even with great care some silica was always pulled up into the syringe as well. Therefore the sample response was that for the solute in 10 μ l of mobile phase and stationary phase and not just for the mobile phase. This will cause an overestimation of the amount of solute in the mobile phase. It is quite likely that all of the solute was not available for partitioning into the bonded alkyl chains of the stationary phase since some of it could have been entrapped in the silica pores or adsorbed onto the interior of the volumetric flask. The phase ratios for the dynamic equilibrium systems were 0.01609 and 0.02297 for the benzene and naphthalene systems respectively; in the chromatographic column the phase ratio was 0.1897. The phase ratio in the dynamic system of necessity had to be much smaller (larger V_m) in order to be able to stir the mixture. Therefore there was ten times more mobile phase in the dynamic system than in the chromatographic one, and this will lead to a larger amount of solute partitioning into mobile phase in the dynamic system than in the chromatographic system, causing a lower value for the partition coefficient.

Finally, it was predicted in Chapter IV that the chromatographic partition coefficient was probably overestimated since the solvation layer volume could not be accurately estimated. This would result in too small a value for V_s and too large a value of V_m , resulting overall in an overestimation of $K_{\text{chromatographic}}$. The comparison of the results for the benzene system are encouraging in this respect; there is about a factor of two difference in the chromatographic and dynamic partition coefficients and this is quite good when all sources of error are considered. The acetonitrile system showed a much larger discrepancy, but this is not unexpected. The k' values in the 85/15 acetonitrile/water system have much more error associated in their measurement than in the methanolic mobile system since the retention times in the acetonitrile system were shorter for the solute (less than 1.5 minutes) as was the system "dead time" due to a thicker solvation layer in acetonitrile systems than in methanolic ones (McCormick and Karger, 1980). Additionally, the molar amount of naphthalene in the acetonitrile dynamic system was eight times less (and the mass amount 13 times less) than the amount of benzene put into the methanolic system; this was necessary because of the large differences in their molar absorptivities at 254 nm. If the absolute amount of error is constant in both systems, the relative amount of error in the naphthalene system will be much larger than that in the benzene system due to the much smaller amount of solute involved. In

conclusion, the results of the dynamic equilibrium experiments show that the chromatographic method of determining partition coefficients may hold great promise in systems with moderate to long (> 5 minutes) retention times, especially if the solvation layer volume can be accounted for accurately.

The Effect of Octadecyl Bonding Density on the
Chromatographic Partition Coefficient

Examination of Tables 4-4, 4-5 and 4-6 and their corresponding figures (4-1, 4-2 and 4-3) shows that for a small nonpolar solute like naphthalene in a methanol/water or acetonitrile/water mobile phase system, solute retention (as measured by its partition coefficient) increases linearly until a bonding density of about $3.1 \mu\text{mol}/\text{m}^2$ is reached. At bonding densities higher than this, the partition coefficient begins to decrease as the bonding density increases. Although errors in the absolute value of the partition coefficient (due to ignoring the solvation layer volume) have been discussed in the previous section as well as in Chapter IV, these unidirectional errors will result in an overestimation of the partition coefficient by a relatively constant amount. As mentioned in Chapter IV, this will cause the curves in Figures 4-1 through 4-3 to be shifted vertically by a constant amount, but the shape of the plot and the trends exhibited by it as well as its maximum will be unchanged.

The retention trends in these plots are best examined in light of Dill's (1987a and 1987b) theory of RPLC retention, which was discussed in Chapter I. Our

experimental plots of partition coefficient versus bonding density correlate very well with Dill's predictions. The low density region which was predicted to be from about 0 to $2.7 \mu\text{mol}/\text{m}^2$ (Dill, 1987b) occurs from 0 to about $3.1 \mu\text{mol}/\text{m}^2$ in our plots. In this region, solute retention increases linearly with increasing alkyl surface density because at low alkyl densities configurational constraints are very small and therefore chain packing has no effect on solute retention. Nonpolar solute retention, as exemplified by the naphthalene solute, increases as the chain volume increases since there is more alkyl chain volume for the solute to partition into. Dill predicted that the y-intercept of this linear region, where octadecyl bonding density was zero, would also occur at zero (no solute retention), yet all of our plots gave nonzero intercepts. There are two theories to explain this effect--either the naphthalene solute exhibited a small amount of adsorption behavior on the silica in addition to its partitioning behavior into the alkyl chains, or the nonzero intercept was a result of additional solute retention on the bonded trimethylsilyl groups used to deactivate the low density bonded phases. In order to decide which theory was correct, naphthalene retention on a bare silica column and a trimethylsilyl (TMS) column was examined. In both mobile phase systems at both temperatures, the naphthalene solute was completely unretained on the bare silica column. In contrast, using the TMS column with both mobile phase systems at both

temperatures resulted in naphthalene being slightly retained ($k' = 1.15$ for 55/45 methanol/water at 35.0 °C and 1.42 at 20.0 °C; $k' = 0.148$ for 85/15 acetonitrile/water at 35.0 °C). The corresponding chromatographic partition coefficients for 55/45 methanol/water at 35.0 °C and 20.0 °C and for 85/15 acetonitrile/water at 35.0 °C are 21.9, 26.8 and 2.79; the respective intercept values for the linear regions of the plots are 12.7, 12.3 and 1.54. It is not surprising that the partition coefficients for naphthalene on the TMS column are much larger than the plot intercepts since the C_1 bonding densities for the low density bonded phases ranged from 0.63 to 1.90 $\mu\text{mol}/\text{m}^2$ and that for the TMS column was 3.16 $\mu\text{mol}/\text{m}^2$; it would be expected that partition coefficient would increase with bonding density for the bonded TMS groups since chain ordering could not occur. Therefore the nonzero intercept behavior is due to the presence of the trimethylsilyl groups in the low density columns. Also note that the acetonitrile mobile phase system results in a much smaller slope in the linear region than the methanolic system. This can be explained (as in Chapter V with selectivity plots) by the more robust nature of the acetonitrile solvation layer; changes in bonding density will affect retention to a much smaller extent than in the methanolic systems, where the mobile phase solvation is not so extensive.

In the high bonding density region (greater than 3.1 $\mu\text{mol}/\text{m}^2$) where bonding densities have surpassed the

projected critical bonding density predicted by Dill (1987a) the partition coefficient definitively decreases with increasing bonding density, just as Dill's model had predicted. At these high bonding densities packing constraints are severe, and as the densities get higher more and more energy is required to create a solute sized cavity in the interphase structure. Since in the Dill model (1987a) the driving force for solute retention is the creation of this interphase solute cavity, the chromatographic partition coefficient for the solute (and hence its retention) decreases due to the increasing amount of energy necessary for cavity formation. If we were able to synthesize extremely high density phases (about $8 \mu\text{mol}/\text{m}^2$), Dill (1987a) predicts that solute retention would no longer occur since the solute would be energetically unable to penetrate the packing structure.

The exhibited close correlation between Dill's predictions (1987a and 1987b) and our experimental data leaves little doubt that partitioning is the dominant mode of RPLC retention. Were Melander and Horvath's (1980) "solvophobic" model true, retention would be unaffected by the surface chain density; in contrast partitioning is strongly affected by this parameter. The curious reader is probably wondering why this retention behavior has never been noticed prior to now. Recall that the most widely used parameter for the examination of retention behavior is the capacity factor, k' , which is the product of the partition

coefficient and the volume phase ratio (stationary/mobile). Examination of the partition coefficients in Tables 4-4, 4-5 and 4-6 shows that they decrease in very small increments for bonding densities ranging from 3.15 to 3.60 $\mu\text{mol}/\text{m}^2$. However, at these same bonding densities, V_s has increased from 0.3094 cm^3 to 0.3424 cm^3 in this range, while V_m has remained essentially constant. Therefore the cumulative effect of these two changing parameters on k' was for k' to increase very slightly; this increase was so slight that k' essentially remains constant over this bonding density range. To our knowledge, this is the first systematic study wherein the effects of bonding density on retention was examined; our approach is also unique and fundamental because we have deconvoluted the effects of changing phase ratio from the measured chromatographic quantity (k') by examining the thermodynamic partition coefficient, which is the most fundamental parameter involved in solute retention.

One significance of this work is that it gives insight into the RPLC retention process at the molecular level. Such fundamental information is of great importance, since once RPLC retention is fully understood prediction of RPLC retention for new solutes will be facilitated, perhaps eventually leading to a predictive RPLC retention index system. One additional strength of this theory is that it is predictive without the use of adjustable parameters.

In addition to the insight that this work gives on the importance of chain ordering and density on RPLC retention,

this work is also relevant to partitioning behavior in micelles, membranes, vesicles and other organized assemblies. These organized systems are of great physiological importance, especially in terms of studying drug structure and pharmacological activity. It is possible that octanol-water partition coefficients may be better correlated with chromatographic partition coefficients than with $\log k'$. Since the structure of RPLC stationary phases is much more membrane-like than that of the octanol-water system, RPLC retention data has also been used to try to characterize the lipophilic nature of solutes and therefore it has been used as a parameter in the quantitative structure activity relationships that relate drug structures with activity (Braumann, 1986; Carney, 1985; Kaliszan, 1986; Miller et al., 1985). Since in RPLC alkyl densities can be varied, the RPLC systems have an advantage over organized assemblies for these studies. This work may therefore have far reaching significance in understanding the behavior of other organized assemblies as well as in the understanding of RPLC retention.

Suggestions for Future Work

Improved Syntheses of High Alkyl Density Bonded Phases

In light of the high bonding densities that we have been able to achieve at subambient temperatures using ultrasound as a driving force for the synthesis of alkyl derivatized stationary phases, future studies of this technique are warranted. Even lower subambient reaction

temperatures such as -10 or -20 °C should be investigated since lower temperatures will result in still more ordered systems as well as further reducing the reaction solvent vapor pressure, promoting even more efficient ultrasonic cavitation (Suslick, 1986). Along this same line of reasoning, the use of a reaction solvent with a lower vapor pressure than methylene chloride may improve ultrasonic efficiency. Suslick (1986) states that the greater the solvent vapor pressure within the cavitation bubble prior to collapse, the less effective the collapse. He also recommends solvents with low chemical reactivity in order to minimize the solvent concentration in the vapor phase of the cavitation event. Therefore, these ultrasound reactions should be attempted using toluene as the reaction solvent, since it is chemically inert, has a high vapor pressure and is a good solvent in terms of silane solubility.

According to Suslick (1986), sonochemical reactivity is also greatly affected by the ambient gas atmosphere. It is desirable to maximize the temperature reached during microbubble collapse since this is one of the driving forces behind sonocatalysis. The maximum temperature attained during cavitation is strongly dependent on the polytropic ratio (C_p/C_v) of the ambient gas, since this defines the amount of heat released during the adiabatic compression of the gas. Suslick (1986) states that cavitation in the presence of xenon ($C_p/C_v = 1.67$) versus freon ($C_p/C_v = 1.1$) would result in a sevenfold ratio of maximum cavitation

temperatures. For argon or helium, $C_p/C_v = 1.67$ whereas for nitrogen, $C_p/C_v = 1.40$, so an argon or helium atmosphere will result in a higher cavitational temperature than that achieved with nitrogen. He also mentions that nitrogen undergoes redox and radical reactions in the presence of ultrasound. For these reasons, Suslick (1986) advocates the use of helium or argon atmospheres for sonochemical reactions, and the effect of these gases on the ultrasound RP syntheses should certainly be examined.

Although dimethyloctadecylchlorosilane of high purity is commercially available, there are other silane reagents which can be synthesized that could give even higher bonding densities than those achieved using the chlorosilane. Szabo et al. (1984) have reported that octadecyl bonded silicas with bonding densities of $4.18 \mu\text{mol}/\text{m}^2$ have resulted from reaction of silica with dimethyloctadecyl(dimethylamino)silane at 125°C for 120 hours; they also give the reaction scheme for this reagent's synthesis. It is postulated that this silane results in very high bonding densities because the dimethylamino moiety is a better leaving group than chloride (Szabo et al., 1984). Golding et al. (1987) have also synthesized a new silane which results in higher bonding densities. They have synthesized octadecyldihydrochlorosilane; in this silane two hydrogen atoms have replaced the two methyl groups found in the commercially available silane reagent. It is thought that the dihydrosilane leads to higher surface coverages because of

its reduced size; replacing the two bulky methyl groups with hydrogens should greatly reduce steric hindrance at the silica surface. Golding et al. (1987) were able to achieve bonding densities of about $4.6 \mu\text{mol}/\text{m}^2$ using this reagent. It is suggested that one or both of these novel silane reagents be tried in the ultrasound synthesis.

Our optimization of the reaction variables (temperature, reaction time, amounts of reagents used, etc.) has thus far followed the "educated trial and error" method, whereby the optimum conditions cited in the literature were used as a starting point and variables were altered in a unilateral fashion. In order to find the best conditions for ultrasound syntheses, the reaction variables should be optimized systematically using a statistically sound method such as experimental design or simplex optimization. By application of Plackett-Burman matrix statistics, Jones (1987a and 1987b) was able to reduce the number of experiments necessary to optimize the bonding reaction to twenty-four, even though twenty-one variables were involved. Such a study should now be done on the ultrasound synthesis. Besides the variables mentioned above, the effect of higher acoustic power of the ultrasound source should also be examined.

Finally, bonding reactions run at high pressures should be attempted. Synthetic groups at the University of Florida are able to run reactions at pressures of 10,000 atmospheres or more. Bonding reactions run at these high pressures may

result in reversed phases with very high alkyl bonding densities.

Bonded Phase Efficiency Studies

Although the bonding densities for reversed phases synthesized by refluxed and ultrasound methods have been compared, their chromatographic efficiencies have not. Chromatographic efficiency is expressed as the number of theoretical plates, N , or as the reduced plate height, h . Column efficiency is evaluated by measuring the plate height for a solute at a number of mobile phase flow rates; plate height is plotted versus mobile phase linear velocity to obtain a van Deemter plot (Snyder and Kirkland, 1979). It would be interesting to compare column efficiencies for two bonded phases (one prepared by ultrasonic methods and the other by reflux) of comparable octadecyl bonding density. We have postulated that the bonded alkyl groups of ultrasonically synthesized stationary phases may be distributed more homogeneously than those of refluxed phases; this could result from increased pore penetration by the silane reagent under ultrasonic conditions. If this is the case, a more homogeneous distribution of bonded alkyl groups should effect a more homogeneous energy of transfer of the solute between the mobile and stationary phases. If this occurs, the corresponding chromatographic peak should exhibit a narrower and more Gaussian distribution, resulting in a lower (improved) reduced plate height. These efficiency comparisons between the ultrasound and refluxed

phases have practical significance, as enhanced column efficiencies bring about improved resolution between chromatographic peaks, an important consideration in difficult separations. Efficiency comparisons between ultrasonic phases of average (around $2.8 \mu\text{mol}/\text{m}^2$) and high (around $3.6 \mu\text{mol}/\text{m}^2$) bonding densities should also be carried out, in order to see if the higher bonding density phases have a correspondingly higher resistance to mass transfer and thus a lower chromatographic efficiency. Sander and Wise (1987) have noted that column efficiency degrades with increased bonding density for polymeric stationary phases; it is important that monomeric phases be evaluated in the same manner.

As explained in Chapter IV, stationary phases of lower octadecyl bonding density were synthesized by "pre-endcapping" the silica with a less than stoichiometric amount of trimethylchlorosilane (TMCS) prior to exhaustive octadecylation. Marshall et al. (1984 and 1986) have reported that the "pre-endcapping" treatment results in more efficient stationary phases as well as significant reductions in peak tailing. This is explained in terms of increased homogeneity of the bonded octadecyl groups. The TMCS is postulated to bond at the most reactive silanol sites, deactivating them and creating a more homogeneous silanol distribution for reaction with the octadecyl silane (Marshall et al., 1984 and 1986). Since we have synthesized a number of these "pre-endcapped" phases, it would be

illuminating to see if we obtain the same results as Marshall et al. as well as to study the efficiency of these phases as a function of degree of "pre-endcapping." Such studies can supply additional information on the prevalence of reactive silanols on the silica surface.

Further Retention and Selectivity Studies

Once bonded phases of even higher alkyl densities ($> 3.6 \mu\text{mol}/\text{m}^2$) can be synthesized, they must be examined in terms of both chromatographic retention and selectivity. The partition coefficients of small solutes on these very high density phases should be evaluated in order to further test Dill's (1987a) predictions. These partition coefficient experiments (as described in Chapter IV) should also be carried out with different types of solutes: large molecules (such as PAHs) as well as those with different types of shapes such as rodlike, chainlike, planar and nonplanar molecules. This will illuminate whether the relationship between bonding density and partition coefficient predicted by Dill (1987a) and demonstrated in our experiments with small molecules will also hold true for larger molecules of different shapes. These experiments should provide additional fundamental information about the nature and organization of the bonded interphase region. Since a change in mobile phase composition will affect chain ordering by changing the stationary phase solvation layer, partition coefficient experiments (as described above) performed under different mobile phase conditions should

give additional information about bonded chain organization under these conditions.

The use of ^{13}C enriched methanol and acetonitrile and D_2O mobile phases in conjunction with ^{13}C and ^2H FT-NMR experiments could also be utilized in order to quantitate the stationary phase solvation layer. This would give information about the solvation structure as well as lead to a more accurate determination of the column phase ratio, since the solvation layer volume could now be added to the bonded chain volume (as calculated in Chapter IV) to get the total stationary phase volume. The solvation layer volume could also be subtracted from the maximum mobile phase volume (as determined in Chapter IV) to obtain a more accurate V_m value; the combination of these two effects would be a very accurate determination of the phase ratio and capacity factor and therefore of chromatographic partition coefficients. Carbon-13 FT-NMR spin lattice relaxation time (T_1) experiments to study chain mobility on bonded phases have been carried out by other workers (Bayer et al., 1986; Gangoda and Gilpin, 1983; Gilpin and Gangoda, 1984; Shah et al., 1987) but the effect of bonding density on chain mobility should also be explored. Additionally, temperature effects on mobility and retention should be studied over a wide temperature range using solution state ^{13}C FT-NMR and liquid chromatographic measurements. It would be particularly interesting to see if there is a certain "critical" temperature at which the retention or

mobility behavior exhibits an abrupt change due to a conformational change in the interphase structure. Such changes in reversed phase behavior have been noted in gas phase experiments (Claudy et al., 1985; Gilpin, 1984; Gonnet, et al., 1985) but have not been reported under true liquid chromatographic conditions.

Selectivity experiments (such as described in Chapter V) should also be carried out with large molecules such as PAHs as well as with solutes of different shapes: rodlike, chainlike, planar and nonplanar. Although such experiments have been performed (Lochmuller et al., 1985; Tanaka et al., 1982; Wise and Sander, 1985) they too have not been carried out on monomeric stationary phases of varying octadecyl density; therefore the effects of chain ordering on selectivity has not been fully explored for these different types of molecules. Again, selectivities for these compounds using mobile phases of different compositions should provide information on stationary phase structure under different mobile phase conditions. As mentioned in Chapter V selectivity experiments actually provide more information about stationary phase structure than retention experiments; since they are measures of retention differences of solutes they are unaffected by the column phase ratio (Antle and Snyder, 1984; Colin et al., 1983a and 1983b). Selectivity behavior of these solutes at different temperatures should be examined as well. In particular, selectivity behavior at subambient temperatures should be

evaluated since the separation factor α for two solutes increases with decreasing temperature (Snyder, 1979). It is likely that this effect is due to a more ordered bonded phase structure at lower temperatures. These selectivity studies would have great practical significance as well, because the most effective way to increase the resolution of difficult to separate compounds is to increase chromatographic selectivity.

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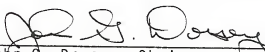
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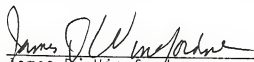
BIOGRAPHICAL SKETCH

Karen Belinda Sentell was born in Charleston, South Carolina, on January 28, 1957. She attended Brentwood Elementary School and Gordon H. Garrett High School (both in Charleston Heights, South Carolina), graduating from Gordon H. Garrett High School in June, 1974. She lived and worked in Charleston, South Carolina, until September, 1979, when she moved to Columbia, South Carolina. She entered the University of South Carolina (Columbia, South Carolina) in January, 1980, receiving her B.S. in chemistry (Magna Cum Laude) in December, 1982. She entered graduate school at the University of Florida in January, 1983, where she was awarded a University of Florida Women's Fellowship. She was awarded two American Chemical Society Analytical Division Graduate Fellowships--a summer fellowship for 1985 and a full-year fellowship for 1986-1987. Upon completion of the requirements for the degree of Doctor of Philosophy (December, 1987) she served as a Leopold Schepp Foundation Postdoctoral Fellow at the University of Florida under Dr. John G. Dorsey.


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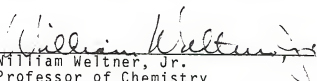
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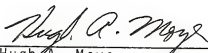
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